

University of Massachusetts Amherst ScholarWorks@UMass Amherst

Open Access Dissertations

9-2013

Performance and Signaling in the Green Anole Lizard

Justin P. Henningsen

University of Massachusetts Amherst, justin.henningsen@gmail.com

Follow this and additional works at: https://scholarworks.umass.edu/open_access_dissertations

Part of the [Other Animal Sciences Commons](#), [Physiology Commons](#), and the [Zoology Commons](#)

Recommended Citation

Henningsen, Justin P., "Performance and Signaling in the Green Anole Lizard" (2013). *Open Access Dissertations*. 799.
<https://doi.org/10.7275/93aw-8d48> https://scholarworks.umass.edu/open_access_dissertations/799

This Open Access Dissertation is brought to you for free and open access by ScholarWorks@UMass Amherst. It has been accepted for inclusion in Open Access Dissertations by an authorized administrator of ScholarWorks@UMass Amherst. For more information, please contact scholarworks@library.umass.edu.

Performance and Signaling in the Green Anole Lizard

A Dissertation Presented

by

JUSTIN P. HENNINGSEN

Submitted to the Graduate School of the
University of Massachusetts Amherst in partial fulfillment
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

September 2013

Organismic & Evolutionary Biology

Performance and Signaling in the Green Anole Lizard

A Dissertation Presented

By

JUSTIN P. HENNINGSEN

Approved as to style and content by:

Duncan Irschick, Chair

Elizabeth Jakob, Member

Stephen McCormick, Member

Jeffrey Podos, Member

Elizabeth R. Dumont, Director
Organismic & Evolutionary Biology

DEDICATION

For Kristin.

ABSTRACT

PERFORMANCE AND SIGNALS IN THE GREEN ANOLE LIZARD

SEPTEMBER 2013

JUSTIN P. HENNINGSSEN, B.S., NORTHERN ARIZONA UNIVERSITY

Ph.D., UNIVERSITY OF MASSACHUSETTS AMHERST

Directed by: Professor Duncan J. Irschick

Green anoles are small lizards of the Southeastern United States. They possess an extensible throat-fan called a dewlap. Males have larger dewlaps and display them more often than females. Displays occur during courtship, during agonistic interaction, and during encounters with potential predators. The size of the dewlap of male green anoles is positively correlated with maximum bite force capacity. Bite force capacity, in turn, is predictive of the winner during agonistic interactions. The correlation between these traits suggests that dewlap size may be used as a reliable signal of maximum bite force capacity. In this dissertation, I address three components of this system. First, I manipulate dewlap size and stage interactions between male green anoles. By removing the correlation between dewlap size and bite force, I was able to show that during staged encounters, bite force is predictive of the winner, but dewlap size is not. In the second chapter, I test for potential costs due to an increased predation risk as a result of dewlap displays. I observed no difference in recapture rates between animals that were experimentally prevented from extending their dewlaps and control counterparts. However, other data suggest that the presence of a pink dewlap may increase risk of predation. These results suggest that though there may be a predation-based cost to dewlap displays, green anoles use behavioral means to ameliorate this risk. Finally, I use

a castration and hormone-replacement experiment to test whether testosterone mediates seasonal changes in dewlap size and bite force. I hypothesized that testosterone would mediate changes in both traits in order to maintain the correlation between them. However, I found no effect of testosterone on change in dewlap size or bite force performance. Another trait, sprint speed performance, was affected by hormone treatment in the breeding season such that animals with high levels of testosterone ran faster than animals from the other treatment groups. My results corroborate other research showing that hormones regulate plasticity in some systems, while other systems are insensitive. However, the mechanisms that regulate changes in the two components of the reliable signaling system in green anoles remain unknown.

TABLE OF CONTENTS

	Page
ABSTRACT.....	iii
LIST OF TABLES.....	viii
LIST OF FIGURES.....	ix
CHAPTER	
1. AN EXPERIMENTAL TEST OF THE EFFECT OF SIGNAL SIZE AND PERFORMANCE CAPACITY ON DOMINANCE IN THE GREEN ANOLE LIZARD.....	1
2. TESTING FOR POTENTIAL PREDATION COSTS OF A RELIABLE SIGNAL....	23
3. ARE SEASONAL CHANGES IN A PERFORMANCE TRAIT AND ITS RELIABLE SIGNAL MEDIATED BY TESTOSTERONE? EXPERIMENTS UNDER BREEDING AND NON-BREEDING CONDITIONS.....	38
BIBLIOGRAPHY.....	62

LIST OF TABLES

Table	Page
1.1: Results of paired <i>t</i> -tests of three variables between winners and losers of staged encounters. Bite force is measured in Newtons, mass in grams, and dewlap size in centimeters ² . We include the mean difference of each variable and their 95% confidence intervals in order to assess biological relevance. Positive numbers indicate larger values for winners.....	19
2.1 Model parameters describing the probability of recapture.....	35
2.2 The number of clay models showing evidence of predation attempts.....	35
3.1 Descriptive statistics of the data measured in each experiment.....	56
3.2 Non-breeding season experiment.....	57
3.3 Breeding season experiment.....	58

LIST OF FIGURES

Figure	Page
1.1: A single individual shown before (A) and after (B) dewlap reduction. The black line in B shows the original outline of the dewlap. The dewlap of this individual was decreased by 27%.....	20
1.2: Histogram of snout-vent length of mature males from this population near Aiken, SC, U.S.A. Note the lack of a trough between 63 and 64 mm, as seen in Lailvaux et al. (2004).....	21
1.3: Number of behaviors by focal males during staged encounters. HB/PU is a headbob or pushup; other variables are defined in the text. The line shows the median value, the box edge is at the 25 th and 75 th percentile, and the whiskers are at the 10 th and 90 th percentile. Individuals outside of these quantiles are shown separately.....	22
2.1 An X-ray of <i>Anolis carolinensis</i> head and dewlap anatomy. The thick white line distal to the junction of the basihyal and ceratobranchial 1 shows the location where the second ceratobranchial 2 cartilages were severed. Image courtesy Beth Brainerd.....	36
2.2 (A) Representatives of the three types of clay models used. From left to right: Pink Dewlap, Green Dewlap, No Dewlap. (B) A model with typical marks from a predation attempt.....	37

3.1: Plasma testosterone levels four weeks after treatment for (a) the breeding season experiment, (b) the non-breeding season experiment. Whiskers are Standard Errors of the Mean.....	59
3.2: Box and whisker plots of four variables for (a) the breeding season experiment and (b) the non-breeding season experiment. Central black lines show the median, the edges of the boxes are the 25 th and 75 th percentiles, and the ends of the lines are the minima or maxima within 1.5 times the interquartile range. Values outside this range are represented by individual points.....	60
3.3: Box and whisker plot of maximum sprint speed during the breeding season experiment. Boxplot elements are the same as Figure 3.2.....	61

CHAPTER 1
AN EXPERIMENTAL TEST OF THE EFFECT OF SIGNAL SIZE AND
PERFORMANCE CAPACITY ON DOMINANCE IN THE GREEN ANOLE
LIZARD

One of the most perplexing issues in animal behavior concerns how animals resolve conflicts (Maynard-Smith & Harper, 2003; Searcy & Nowicki, 2005; Arnott & Elwood, 2009; Briffa & Sneddon, 2010). Rather than resorting to potentially destructive physical force, competitors often settle disputes using a display of signals. Signals are behavioral or morphological traits that influence the behavior of other individuals and may advertise a trait of the signaler such as size, condition, or many other traits (Dawkins & Krebs, 1978; Johnstone, 1997; Searcy & Nowicki, 2005). If a trait is consistently correlated with a component of the signal (e.g., size, frequency, color; hereafter referred to as intensity), the signal is said to be reliable (e.g., Johnstone, 1997; Searcy & Nowicki, 2005).

Recent attention in this area concerns the relationship between signals and whole-organism performance traits, such as maximum sprinting ability, bite force, etc. (Irschick *et al.*, 2007; Byers, Hebets & Podos, 2010). Whole-organism performance traits represent the integrated output of lower-order systems (e.g., muscles, biochemistry, etc.) and can be under strong natural (Arnold, 1983; Irschick *et al.*, 2008) and sexual selection (Lailvaux & Irschick, 2006a; Husak, Lappin & Van Den Bussche, 2009; Byers *et al.*, 2010). Studies in taxa as diverse as lizards, beetles, and crustaceans show that individual variation in performance capacity is often a strong predictor of dominance during conflicts (e.g., Robson & Miles, 2000; Sneddon *et al.*, 2000; Briffa, Elwood & Russ, 2003; Perry *et al.*,

2004; Huyghe *et al.*, 2005; Lappin & Husak, 2005). This work demonstrates that quantifying performance capacity can provide insight into the mechanisms determining the outcome of interactions between fighting individuals (reviewed in Lailvaux & Irschick, 2006a). Furthermore, in at least some of these cases, there is evidence that the intensity of the signal acts as a reliable indicator of whole-organism performance capacity involved in resolving fights (Vanhooeydonck *et al.*, 2005b).

However, these studies are primarily descriptive, and for the most part have not experimentally manipulated either animal performance or signal size (but see Adams & Caldwell, 1990; Briffa & Elwood, 2000; Tibbetts & Lindsay, 2008). Because reliable signals are by definition correlated with a second trait, it is challenging to determine whether the signal or the correlated trait determines the outcome of an interaction without first decoupling the trait and the signal. Although signals and whole-organism performance traits are important influences on the outcome of male fights, in some circumstances other variables may play an even greater role. For many animals, body size is the most important predictor of fight success, with larger individuals having a distinct advantage (e.g., Clutton-Brock *et al.*, 1979; Whitham, 1986; Hughes, 1996; Wikelski & Trillmich, 1997; Karsten *et al.*, 2009). A second well-studied factor is body condition, which has received a great deal of attention (reviewed in Jakob, Marshall & Uetz, 1996; Peig & Green, 2010). Condition has been examined as a factor in male competition (Hack, 1997; Fitzstephens & Getty, 2000; Jonart, Hill & Badyaev, 2007), but few studies have asked how both condition and physical performance influence fight success. In sum, researchers have investigated how animals resolve conflicts using many different variables, but few studies have taken a manipulative approach to determine how

variation in whole-organism performance capacity, signal size, and condition together influence fight success. Here, we take such an approach by experimentally manipulating a signal (the anole dewlap) and examining the effect of the signal, performance capacity, and body condition on dominance in male green anole lizards (*Anolis carolinensis*).

We chose to use green anoles to explore the relationships among signal size, whole-organism performance, condition, and male dominance for several reasons. First, this lizard has been widely studied in terms of its behavior (Decourcy & Jenssen, 1994; Jenssen, Greenberg & Novde, 1995; Lailvaux *et al.*, 2004), and this background information allows us to more easily interpret our results. Second, male green anoles are highly territorial and display an extendable throat fan, or dewlap, during encounters with other males, during courtship with females, and during non-directed displays (Greenberg & Noble, 1944; Jenssen *et al.*, 1995). Third, the size of the dewlap in male green anoles has been shown to be a reliable signal of maximum bite force in some size classes and/or populations, even when each is corrected for body size (Vanhooydonck *et al.*, 2005b; Irschick *et al.*, 2006; Lailvaux & Irschick, 2007). Finally, winners of staged encounters between large size-matched males have significantly higher bite forces than losers in at least one population (Lailvaux *et al.*, 2004). Thus, in green anoles, the dewlap appears to reliably signal a trait important to the outcome of dominance contests, at least in the populations studied so far. However, none of these studies have manipulated either performance capacity or signal size, and therefore the causal relationship among these variables and dominance remains unclear.

In order to manipulate dewlap size, we have modified the methods of Crews (1975), Tokarz (2002) and Tokarz *et al.* (2003, 2005), who disabled dewlap displays by

severing the second ceratobranchial cartilages of male green and brown (*A. sagrei*) anoles, respectively. This treatment strictly prevented the male from extending the dewlap to any extent, but left all other aspects of social behavior unaltered. While these studies provided useful data on dewlap function, it is generally rare for free-ranging green anole males to exhibit such an extreme impairment (J.P. Henningsen, pers. obs). Our goal was to manipulate the size of the dewlap within biologically meaningful bounds of variation, which are substantial. For example, among the green anole males used in this experiment that were between 65 and 68 mm SVL, dewlap size ranged from 20.9 cm² to 37.3 cm². Hence, rather than disabling dewlap displays entirely, we reduced the size of the dewlap in order to decouple the correlation between dewlap size and bite force, while still allowing the animal to extend the dewlap. We used this manipulation to test the role of dewlap size, bite force, and condition on the outcome of staged interactions between green anole males.

First, we examined the relationships among bite force, dewlap size, and mass in a large group of sexually mature male anoles from our population. Next, we tested the following hypothesis in a subset of the above animals: If male green anoles use dewlap size to assess performance capacity during agonistic encounters, animals with surgically reduced dewlaps should win significantly fewer interactions than control animals. Because dewlap size is a reliable signal of bite force performance, animals with reduced dewlaps should be perceived as having lower bite forces than control animals. We predict that control animals will, in turn, be more willing to escalate encounters and will thus be dominant over individuals with reduced dewlaps. Evaluating the relative importance of the anoline dewlap and related performance traits will provide a basis for future work into

the broader ecological adaptive radiation of anoles, in which behavior, performance capacity, and dewlap function vary in their use during male conflicts (Lailvaux & Irschick, 2007).

Methods

Signal Reliability

We captured 243 sexually mature (SVL range: 46.2-69.8 mm; mean: 61.0 mm; sd: 4.30) males from our study population on the Savannah River Site near Aiken, South Carolina, USA during the peak breeding months of April, May, and June in 2009 and 2010. On the day of capture we measured body mass to the nearest 0.01 g with a digital balance (Mettler AE163, Columbus, OH), and snout-vent length (SVL) to the nearest 0.1 mm with digital calipers (Mitutoyo CD-8 CS, Aurora, IL). We measured dewlap size by photographing animals held against a tabletop after extending the dewlap maximally with forceps. We used these images to measure dewlap area with ImageJ (NIH, Bethesda, MD). This method provides reliable and repeatable measurements of dewlap area (Vanhooydonck *et al.*, 2005b). We measured maximum bite force by inducing the animals to bite on the padded ends of steel bite plates connected to an isometric Kistler force transducer (type 9023, Kistler Inc. Wintherthur, Switzerland) connected to a Kistler charge amplifier (type 5058a, Kistler Inc.). Each animal bit five times in two sessions (two or three bites per session) with at least one hour of rest between sessions. We used the maximum of these bites for subsequent analyses (Herrel, De Gruaw & Lemos-Espinal, 2001; Lailvaux *et al.*, 2004).

We tested the significance of the Pearson product correlation between body size, dewlap size, and bite force in order to elucidate the relationships between these traits in animals from this population. We then tested the correlations among dewlap size, bite force, and body mass independent of body size using the residuals of least-squares regressions of dewlap size, bite force, and mass respectively on snout-vent length.

Staged Encounter Animals

Adult male green anoles were collected for staged encounters between May 1 and June 30, 2009. The location of capture was noted so that we could avoid staging encounters between animals that had interacted previously. Specifically, animals captured within 20 m of one another were not used in the same encounter. Anoles were taken to the Savannah River Ecology Lab where we measured morphological traits and bite force as described above. Animals were temporarily housed individually in 13 L (32 x 20 x 20 cm) plastic aquaria. Each cage contained a wooden perch and was lit and heated by overhead lights on a 14:10 h light:dark cycle. Males were size-matched to ± 3 mm SVL (following Robson & Miles, 2000; Lailvaux *et al.*, 2004), and we randomly chose one member of each pair of males to have its dewlap surgically reduced. The remaining individual received a sham surgery (see below). All animals were permanently marked with colored subcutaneous elastomer implants (Northwest Marine Technologies, Shaw Island, WA) on the ventral limb surface.

Surgical Treatments

We anesthetized each lizard with a subcutaneous injection of the local anesthetic lidocaine (1mg/kg) in the dewlap. During the procedure, animals were held in place with surgical tape on a partially thawed chemical ice pack. We made a small incision in the skin of the dewlap on the animal's right side to expose the second ceratobranchial cartilages. In animals assigned to the treatment group, we severed the cartilages at the anterior and ventral edge of the dewlap at approximately one third of the length of the cartilages, and in animals in the control group we probed the cartilages but left them intact. This method differs from previous dewlap manipulations (Crews, 1975; Tokarz, 2002; Tokarz *et al.*, 2003, 2005), which severed the cartilages just distal to the articulation of the second ceratobranchials and the basihyal (see Bels, 1990 for dewlap anatomy). These previous manipulations thus rendered the second ceratobranchials useless. Our manipulation, in contrast, left a portion of the cartilage in a functional state and thus allowed the anole to use the same musculo-skeletal elements to extend the dewlap, but prevented the full extent of the dewlap skin to be displayed (Figure 1). For both treatments, the incision was closed with a tissue adhesive (Vetbond, 3M, St. Paul, MN) and the animal was returned to its cage to recover. All individuals resumed normal behaviors, including basking and feeding, typically within minutes. Each animal was given 24 hours to recover before it was used in an encounter.

Staged Encounters

We staged 48 interactions between 96 individuals using 38 L glass aquarium as a test arena. We covered the back and sides of the arena with an opaque backing. Each end of the arena was separated from a central chamber by two dividers, one of opaque

pressboard and the second of transparent Plexiglas. Each outer chamber contained a brick (6 x 8 x 19 cm) to provide a raised platform for displays. All three chambers of the test arena were approximately equal in size. We chose one random member of the matched pair (either with manipulated dewlap or sham treatment) to be the focal animal, and painted a small dot on its back for identification during the trial. We placed matched lizards (one with manipulated dewlap, the other with sham treatment) into randomly chosen and opposite ends of the arena. After a 15-minute acclimation period, we raised the opaque dividers from behind a hide using a pulley system. The transparent dividers remained, allowing the animals to see and display to one another, but unable to physically interact. This period of visual interaction lasted for 10 minutes, and was scored by an observer from behind the hide (see below). At the end of the 10-minute period, the transparent dividers were raised using another pulley. The lizards then were free to move throughout the arena, including the central chamber where a perch was situated under a lamp. Scoring proceeded continuously for 60 minutes from this point. Thus, 70 minutes of behavior was scored for each interaction. The encounter was also recorded with digital video for independent analysis of the visual phase (see below). A single observer (JPH) observed and scored all interactions. Each animal was used in only a single interaction.

Dominance Scoring

We scored dominance interactions following standard protocols for lizards (e.g., Robson & Miles, 2000; Lailvaux *et al.*, 2004). All observed agonistic behaviors were assigned positive scores using the following system: Headbobs and pushups, defined as a bout of rapid up and down movement of the head or body, and dewlap display bouts were

each scored 0.5. Lateral displays, chases, and bites were scored as 1. Lateral displays were defined as an animal turning its body perpendicular to the line of sight of the other animal combined with lateral compression and dorso-ventral expansion. Chases were defined as running toward an opponent. Retreats, defined as running away from an opponent, were scored as -1. The member of the pair that had the higher cumulative score at the end of the observation period was considered the winner.

Statistical Analyses

To test whether our dewlap manipulations resulted in a significant reduction in dewlap size, we used a two-tailed paired *t*-test to compare dewlap size before and after surgical manipulation. To test whether the surgical manipulations changed display behaviors, we used a series of *t*-tests on the focal animals only. Due to the importance of detecting differences in behavior resulting from surgical manipulation, we do not take a statistically conservative approach to multiple comparisons such as Bonferroni corrections, which are overly conservative (Nakagawa, 2004), and would decrease the probability of detecting significant differences in behavior after dewlap manipulation. Here, we are more concerned with the possibility of committing a type II error, and thus our approach is designed to detect differences in behavior if they are manifest.

We used a goodness-of-fit *G*-test to assess whether dewlap manipulation affected the probability of winning or losing dominance interactions. We used the outcome for the focal animals and compared the proportion of interactions won by animals with reduced dewlaps to 0.5, the proportion expected by chance.

To determine which traits best predicted variation in dominance, we used three paired *t*-tests to compare dewlap size, bite force, and mass of winners and losers (as recommended by Briffa & Elwood, 2010). We chose these variables for comparison *a priori* based on our hypothesis that reducing signal size would change the dominance relationship between males as well as previous work showing that dewlap size or bite force are each key predictors of dominance during staged interactions between male anoles (Lailvaux & Irschick, 2007). We included mass because in many taxa, condition indices are created as functions of body size and mass. Since our comparisons are adjusted for variation in body length, the mass term amounts to a metric of body mass relative to body length, or an index of “fat”, a common metric of condition indices (Jakob *et al.*, 1996; Peig & Green, 2010). We also performed identical comparisons using body condition (residuals from regression of mass on SVL; not shown) rather than mass, and the results were unchanged.

In order to test whether focal animals responded differently when interacting over longer distances, we considered the visual phase of all types of interactions independent of the physical interaction. To do this, we scored from the videos each lizard's behavior for the first 5 minutes of each interaction, or until the two animals were within a body length of one another. This five-minute phase began only after each animal had performed a dewlap display. For these analyses, we include only encounters during which both animals performed at least one dewlap display prior to approach to within a body length ($N = 21$). Over the five-minute phase, we calculated a dominance score as described above. We then used a goodness-of-fit *G*-test to determine if reducing the size

of the dewlap affected agonistic behaviors in the initial stage of the interaction. All analyses were performed in R v2.10.1.

Results

The size distribution of sexually mature male green anoles in our population showed a distinct lack of the bimodality described in previous studies in other geographic locations (Figure 2; Lailvaux *et al.*, 2004; Vanhooydonck *et al.*, 2005b). Thus, we do not present data that separate males by size, as there do not appear to be clear size classes (e.g., heavyweight, lightweight) as described in Lailvaux *et al.* (2004). Within our sample of males, there were significant positive correlations between dewlap size and SVL ($r = 0.78$, 95% CI = 0.73 – 0.83; $t = 19.6$, $df = 241$, $p < 0.0001$), between bite force and SVL ($r = 0.76$, 95% CI = 0.70 – 0.81; $t = 17.9$, $df = 241$, $p < 0.0001$), and between mass and SVL ($r = 0.90$, 95% CI = 0.87 – 0.92), $t = 31.2$, $df = 241$, $p < 0.0001$). When we removed the effects of body size, there were significant positive correlations between relative dewlap size and relative bite force ($r = 0.15$, 95% CI = 0.02 – 0.27; $t = 2.3$, $df = 241$, $p = 0.02$), relative bite force and condition ($r = 0.50$, 95% CI = 0.41 – 0.59; $t = 9.1$, $df = 241$, $p < 0.0001$), and relative dewlap size and relative body mass ($r = 0.21$, 95% CI = 0.08 – 0.32; $t = 3.28$, $df = 241$, $p = 0.001$).

Our surgical manipulations significantly reduced dewlap area by an average of 36.8% relative to original area (Figure 1; mean \pm SE before = 26.6 ± 0.7 , after = 16.8 ± 0.5 ; $t = 16.5$, $df = 44$, $p < 0.0001$). The size range before the treatment was 13.1– 35.4 cm² and the size range after reduction was 6.8– 26.6 cm².

In nine of 48 interactions, one or both animals did not perform any dewlap displays. In the remaining 39 interactions, the manipulation did not significantly affect the frequency of any behavior we measured. Animals with reduced dewlaps performed headbobs/pushups ($t = 0.06, p = 0.95$), dewlap displays ($t = -0.07, p = 0.95$) and lateral displays ($t = 1.40, p = 0.17$) as often as control animals. The number of bites ($t = -1.63, p = 0.12$), chases ($t = -1.39, p = 0.18$), and retreats ($t = 0.73, p = 0.47$) also did not differ significantly between the two groups (Figure 3). Contrary to our hypothesis, reducing dewlap size did not influence the outcome of staged interactions. Control animals with intact dewlaps won 17 of 39 interactions, a value not significantly different than chance ($G = 0.64, \chi^2 \text{ d.f.} = 1, p > 0.4$).

A paired t -test further confirms that dewlap size did not play a role in the outcome of the interactions (Table 1). For this test, we excluded the interactions in which one or both individuals did not perform any dewlap displays during the trial. We also excluded two other trials because we failed to measure the size of the reduced dewlap. Bite force was significantly higher in winners than in losers, but mass did not differ between the two groups (Table 1). Each of these latter tests included all 48 interactions.

We also calculated a dominance score for the initial five minutes of interactions in 21 of 39 cases. Control animals had higher dominance scores in nine of these encounters, which was not significantly different than chance ($G=0.43, \chi^2 \text{ d.f.} = 1, p = 0.51$).

Discussion

Our data present three primary results. First, we show that dewlap size is significantly and positively correlated with maximum bite force in our population. This

relationship holds true when each trait is corrected for body size, suggesting that dewlap size may be a reliable signal of bite force capacity. Next, we have provided experimental evidence that reducing signal size in a controlled setting did not change the likelihood that a male green anole would be dominant during a staged encounter with a size-matched individual. Finally, during the encounters, bite force performance predicts fight success, with males that bite harder being more likely to win.

Overall, our results did not support the hypothesis that a reduction in signal size would cause male lizards to be subordinate to males with intact dewlaps. One possible explanation is that the link between dewlap size and bite force may be most relevant for male green anoles that defend territories by signaling over relatively large distances, a view that is consistent with comparative studies of anole species that vary dramatically in social behavior and dewlap use during fights (Lailvaux & Irschick, 2007). During non-directed territorial displays, male green anoles (and other highly polygynous anoles) actively monitor their territories by regular patrols interspersed with bouts of displays, including dewlapping. We argue that territorial dewlap displays act as clear signals of male size, quality (bite force), and resident status, thus discouraging unseen rival males from intruding. By contrast, during close encounters, more direct features of males such as head size (which is linked to bite force), body condition, or other behavioral traits such as push-ups (linked to endurance in some lizards, Brandt, 2003) may be relevant for assessing opponents, although in this case, only bite force was a significant predictor of fight success. Another possible explanation for the lack of effect of dewlap size is that males in staged encounters are forced to engage in combat regardless of the assessment of an opponent. However, our data do not back this claim. Biting occurred in 23 of 39

(59%) encounters. We analyzed both encounters with biting and those without separately, and our results (not shown) do not qualitatively change. It is notable that relative dewlap size has a rather small positive correlation with relative bite force in our study population ($r \sim 15\%$; somewhat lower than reported in previous studies), and thus cues other than dewlap size may allow rivals further opportunities for assessment. In support of this view, Decourcy and Jenssen (1994) and Jenssen et al. (2000) found that as the distance decreased between males in aggressive encounters, individuals performed fewer dewlap displays, and shifted instead to headbob and pushup displays. As encounters escalated further, green anole males moved closer, lined up laterally and circled one another. Encounters sometimes ended with jaw sparring and biting until one individual withdrew. Qualitatively, we observed a similar sequence of fewer dewlap displays given at close range during our experiment. Another possibility is that dewlap assessment might be most relevant in the initial phases of a fight, but our data from the first five minutes of dewlap displays show that even during the initial interaction that occurred at a greater distance, the response of a focal animal was independent of the dewlap size of the second male. However, it is possible that repeated signaling is required before either contestant relents. Green anoles generally produce displays in volleys (Decourcy & Jenssen, 1994), and repeated displays may allow the displayer the opportunity to reduce error in assessment by an opponent (Enquist & Leimar, 1983; Briffa & Sneddon, 2010). Because we do not know whether or how error reduction is accomplished in these animals, our methods may have failed to elicit or detect this phenomenon, and it remains a topic for future investigations.

Classic theory posits that the winners of male fights in natural settings should have a notable advantage in reproductive success because of increased access to resources, including food, females, refugia, etc. (Warner, Robertson & Leigh, 1975; McCann, 1981; Thornhill, 1981; Andersson, 1994). Such data are scarce on *Anolis* lizards (but see Trivers, 1976), but *A. carolinensis* males actively defend distinct territories that typically contain the territories of several females (Jenssen *et al.*, 1995), and female green anoles are not known to show any pre-copulatory mate choice (Andrews, 1985; Lailvaux & Irschick, 2006b). Hence, the male that controls a territory with one or more females inside it is likely to sire most of the offspring of those females. These facts suggest that green anole males are under strong selection to be dominant over competitors, and our results corroborate this view by suggesting that a high level of whole-organism performance is a strong contributor to male dominance, although other variables may also be important.

The outcome of an aggressive interaction is, in theory, influenced by the difference in participants' resource holding potential (RHP; Parker & Stuart, 1976; Arnott & Elwood, 2008, 2009). There are several lines of evidence to suggest that bite force capacity is an important component of RHP in male green anoles. First, the green anole jaw, like that of many territorial lizard species, is heavily overbuilt for eating, and this excess capacity is likely due to strong selection for biting during male fights (Meyers, Herrel & Birch, 2002; Herrel & Gibb, 2006; Dial, Greene & Irschick, 2008). Second, biting is clearly used as a behavioral strategy to either intimidate or injure opponents, and among anole species, there is a general trend for more polygynous and territorial species to use biting as a strategy to a greater extent compared to less polygynous species

(Lailvaux and Irschick 2007). Therefore, the use of bite force as a component of RHP in male green anoles has a further benefit: in certain cases, functional traits may be related to receiver-dependent costs that enforce signal reliability (Husak *et al.*, In Press). That is, the ability of male green anoles to bite may correlate with its ability to inflict injury to an opponent. If the threat of injury resulting from a bite is substantial, an opponent may be persuaded to give up the contest. (Payne, 1998; Briffa & Elwood, 2009; Briffa & Sneddon, 2010).

The use of body condition as a metric of RHP has entailed considerable attention and controversy (Jakob *et al.*, 1996; Peig & Green, 2010). In some cases, condition is a predictor of fight success (Hack, 1997; Fitzstephens & Getty, 2000; Jonart *et al.*, 2007), and in our trials, there was a non-significant trend for heavier males to win encounters. However, we note that body condition correlates positively with both bite force and dewlap size within our sample of green anoles, which suggests that males with high values of all three traits may be especially vigorous (although many variables contribute to overall vigor, such as immune function, etc.). While we cannot easily dissect these three intercorrelated variables, future studies might be able to manipulate one or all three (e.g., through hormone manipulation or dietary restriction) to gain an understanding of how each independently influences dominance. Other traits could be considered in concert with manipulations of dewlap size, performance, or condition. For example, dominant male green anoles tend to display dark patches behind the eyes more quickly than subordinates, and these eyespots seem to inhibit aggression by opponents. (Summers & Greenberg, 1994; Korzan *et al.*, 2000).

Another future consideration is ontogenetic differences in fighting tactics.

Lailvaux et al. (2004) described a distinct bimodal distribution in the size of adult male green anoles from a population about 20 km from New Orleans, LA, USA. Furthermore, different kinds of performance traits were important for dictating dominance in these two size classes during aggressive encounters, with greater jumping capacity predicting fight success in smaller males, and greater bite force capacity predicting fight success in larger males (Lailvaux *et al.*, 2004). In contrast, the source population for this experiment shows no sign of bimodality (Fig. 2), and the overall relationship between bite force and dewlap size is weaker, though still significant, compared to other populations (Irschick et al. 2006). As noted by Bloch and Irschick (2006), there are substantial geographic differences in social selection pressures among green anole populations, suggesting that male fighting strategies may differ among populations. A more detailed description of geographic variation in population structure would be a fruitful avenue for future study.

Our results show how an experimental approach to signal manipulation can reveal underlying factors that influence male conflict. In this case, destructive performance capacity (bite force) is a better determinant of the outcome of male fights than signal size or body condition. Our results support the general importance of performance traits for influencing dominance interactions and suggest that the link between signals and such traits may be more relevant for general territorial defense, not for resolving conflicts *per se*. Further studies that employ our manipulation techniques could be used to explore models of contest assessment in this species. Additionally, manipulations of dewlap size in free-ranging individuals will be useful for assessing ultimate effects on survival and reproductive success. Such studies might shed light on the utility of the anoline dewlap, a

structure that has proven to be enigmatic, despite many years of study (Losos & Chu, 1998; Ord & Martins, 2006; Nicholson, Harmon & Losos, 2007).

Acknowledgements

This material is based upon work supported by the Department of Energy under Award Number DE-FC-09-075R22506 to the Savannah River Ecology Laboratory, with additional support from the National Science Foundation Graduate Research Fellowship, the Society for Integrative and Comparative Biology Fellowship for Graduate Student Travel, and the University of Massachusetts Natural History Collection Jane Hallenbeck Bemis Endowment for Research in Natural History. We thank A.K. Lappin, B. DeGregorio, J. Podos, E. Jakob, B. Elwood, M. Bee, and two anonymous reviewers for comments on the manuscript, B. Morris and B. DeGregorio for assistance with animal collection, and T. Tuberville for generous logistical support. Animals were collected with South Carolina State permit G-09-03. All work was conducted with approved animal use protocols from University of Massachusetts (#28-10-01) and University of Georgia (#A2009-10092).

Table 1.1: Results of paired t -tests of three variables between winners and losers of staged encounters. Bite force is measured in Newtons, mass in grams, and dewlap size in centimeters². We include the mean difference of each variable and their 95% confidence intervals in order to assess biological relevance. Positive numbers indicate larger values for winners.

Variable	N	t (df)	p	Mean Difference	95% CI
Dewlap Size	37	-0.42 (36)	0.68	-0.79	-4.61 – 3.03
Bite Force	48	2.14 (47)	0.04	0.25	0.02 - 0.49
Mass	48	1.54 (47)	0.13	0.12	-0.04 – 0.28

Figure 1.1: A single individual shown before (A) and after (B) dewlap reduction. The black line in B shows the original outline of the dewlap. The dewlap of this individual was decreased by 27%.

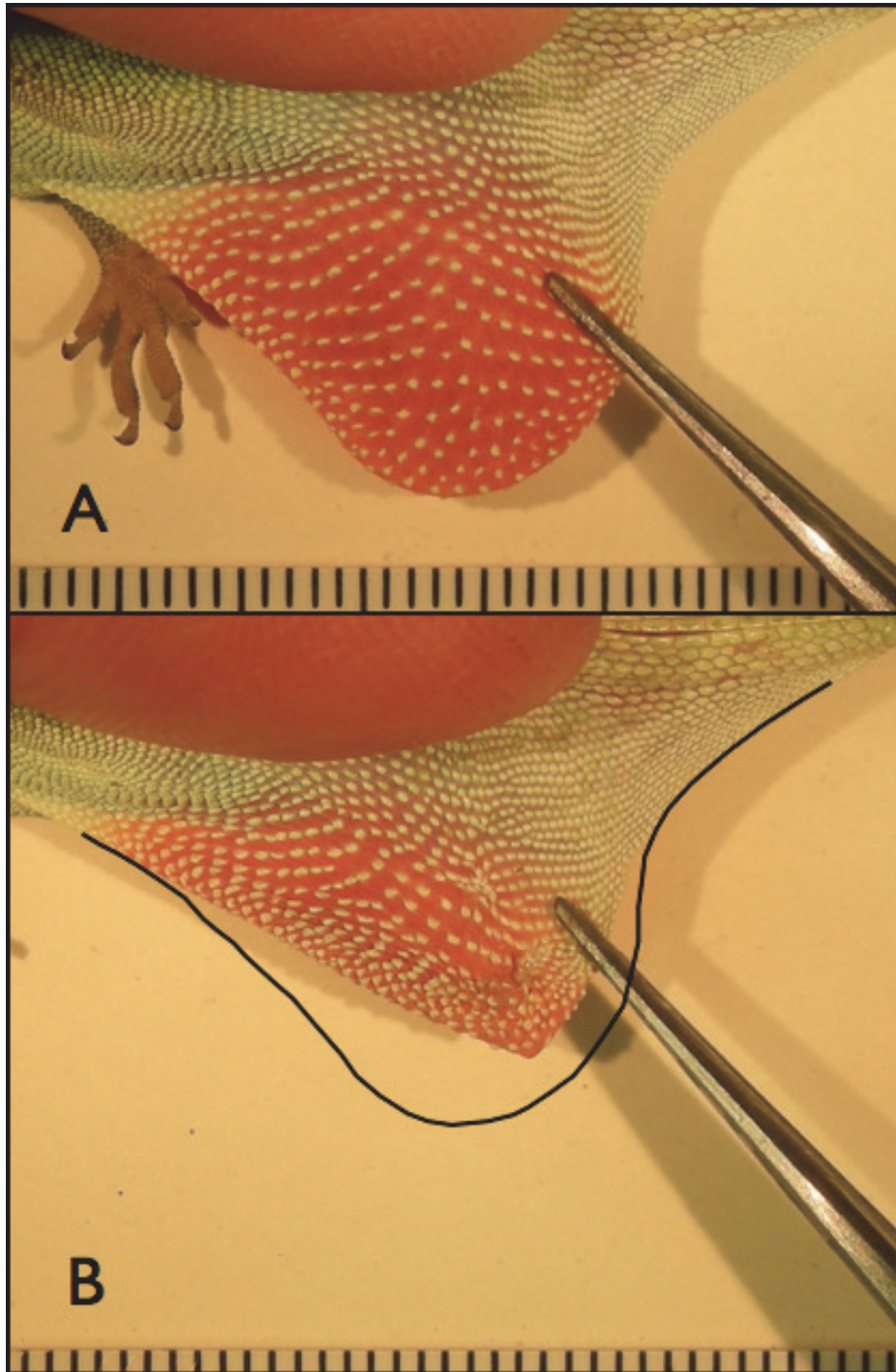


Figure 1.2: Histogram of snout-vent length of mature males from this population near Aiken, SC, U.S.A. Note the lack of a trough between 63 and 64 mm, as seen in Lailvaux et al. (2004).

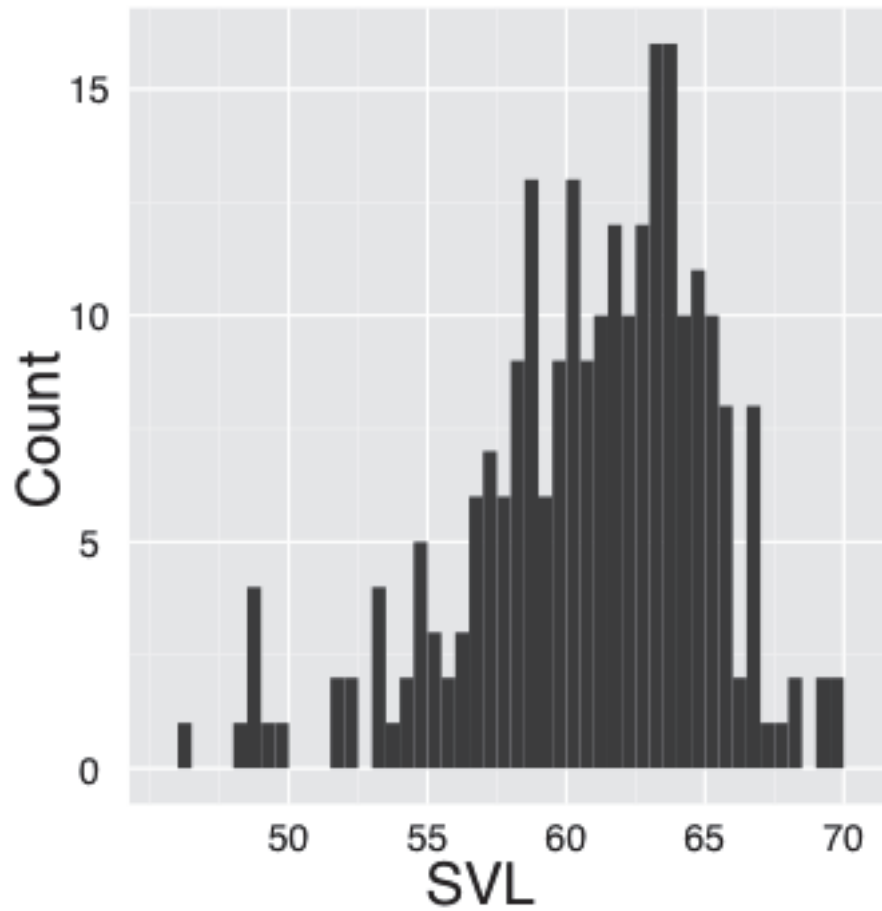
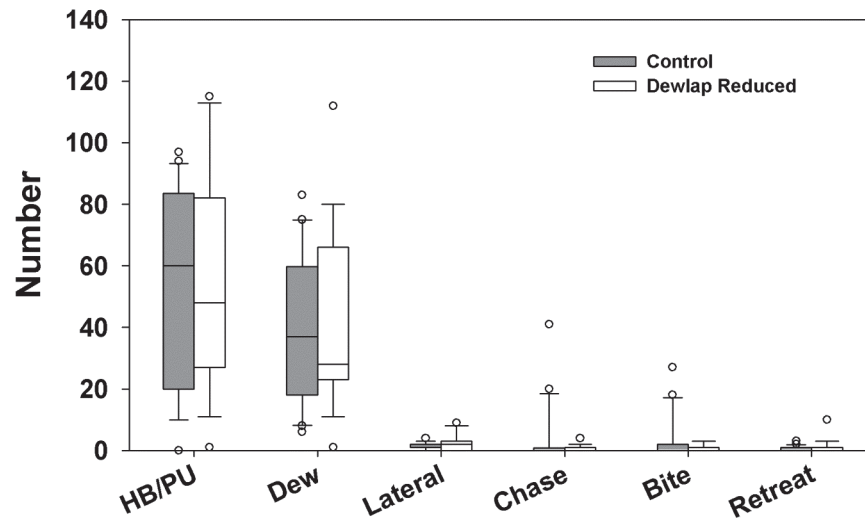


Figure 1.3: Number of behaviors by focal males during staged encounters. HB/PU is a headbob or pushup; other variables are defined in the text. The line shows the median value, the box edge is at the 25th and 75th percentile, and the whiskers are at the 10th and 90th percentile. Individuals outside of these quantiles are shown separately.



CHAPTER 2

TESTING FOR POTENTIAL PREDATION COSTS OF A RELIABLE SIGNAL

Signals are among the most elaborate and distinctive features of many animal taxa. These communication tools are presumably adaptive traits that have evolved to increase the fitness of the signaler (Maynard-Smith & Harper, 2003; Searcy & Nowicki, 2005).

Despite an overall increase in fitness from the signal, evolutionary theory has long predicted the presence of costs in these systems (Zahavi, 1975; Grafen, 1990; Johnstone, 1997). In practice, it has often been difficult to detect such costs, and many studies that look for costs often find only either indirect costs or indirect evidence of viability costs.

Indirect costs include things like increased energy expenditure. Some signals do indeed cost the signaler significant energy output. For example, calling Gray Tree Frogs (*Hyla versicolor*) increase their oxygen consumption to 25 times above resting rates - a value that exceeds consumption even during maximum locomotion (Taigen & Wells, 1985). In other instances, however, seemingly large and burdensome structures do not create energetic demands. Male giant rhinoceros beetles (*Trypoxylus dichotomus*) have long horns used in grappling matches with rivals. Despite their size, these horns do not have a detrimental impact on flight performance, and their energetic costs during flight are negligible (McCullough & Tobalske, 2013). Male stalk-eyed flies have similarly elaborate structures on their heads, which are important in male competition and female choice (Wilkinson, Kahler & Baker, 1998; Panhuis & Wilkinson, 1999). Despite biomechanical predictions of decreased flight performance, Husak et al. (2011) showed a similar lack of costs in stalk-eyed flies, where the elongated eye stalks of males had little impact on flying ability.

In and of themselves, indirect costs such as increased energy expenditure do not necessarily translate to decreased survival or reproductive success (but see Kotiaho, 2000; Hunt *et al.*, 2004). Although it can be valuable to know the energy expenditure associated with a signal, in evolutionary terms, costs are measured in decreased viability or reproductive success (Kotiaho, 2001; Searcy & Nowicki, 2005). Measuring viability costs is more difficult, though such costs have been detected in several taxa. Male Túngara frogs famously include an element in their vocal advertisement that is preferred by female Túngara frogs (Ryan, 1980), but also leaves the male more vulnerable to predation by bats (Ryan, Tuttle & Rand, 1982). Brightly-colored guppies (Endler, 1980) and sticklebacks (Moodie, 1972) are both predated more often than duller counterparts.

The issue of viability costs is further complicated in relation to reliable signals. In instances where an aspect of the signal (hereafter intensity) is consistently and significantly correlated with another traits of the signaler, the signal is said to be reliable (Searcy & Nowicki, 2005). That is, because of the correlation between the signal and the trait, receivers of the signal, on average, gain information about the signaler. In order to prevent cheaters from invading the system by displaying “dishonestly” large traits, theory predicts that the costs enforcing reliability are differential, such that a signal of a given intensity is more costly for an individual of lower quality (Grafen, 1990; Johnstone, 1997; Getty, 1998). Differential costs have been detected in only a few instances. Male barn swallows have elongated tail feathers that are attractive to females, but also make them more vulnerable to predation (Møller & Nielsen, 1997). High-quality males were less likely to be predated than their rivals of lower quality (Møller & Nielsen, 1997). Differential viability costs are also present in wolf spiders, where males that perform a

drumming display at higher rates are targeted more often by predators and experience a drastic increase in metabolic rate that reduces survival. High-quality males, however, withstand both costs better than low quality males as evidenced by increased survival during extreme energy expenditure and increased ability to escape predation attempts (Kotiaho *et al.*, 1998; Lindström *et al.*, 2006).

Green anole lizards (*A. carolinensis*) have a reliable signal with unknown viability costs. The size of the dewlap of male green anoles is positively correlated with maximum bite force capacity (Vanhooydonck *et al.*, 2005b; Irschick *et al.*, 2006; Henningsen & Irschick, 2012). The correlation exists independent of body size such that when two male green anoles interact, the individual with the larger dewlap will, on average, have higher bite force. High bite force is an important trait to this aggressive, territorial, and polygynous lizard because males with a higher bite force tend to win agonistic interactions with individuals possessing lower bite force (Lailvaux *et al.*, 2004; Henningsen & Irschick, 2012). It is worth noting that there is no physiological or mechanical connection between the dewlap anatomy and the muscles responsible for biting. This lack of relationship means this signal is by convention only, and is theoretically open to infiltration by dishonest signalers.

Despite the expectation of a viability cost to dewlap displays, no such costs have ever been measured. Here, we present the results of two experiments designed to test whether dewlap displays may increase the risk of predation. In our first experiment, we surgically disable dewlap displays of free-ranging male green anoles to test the effect of this treatment on recapture rates. We also test a number of other traits related to male quality to determine whether they contribute to survival. We predict that individuals with

disabled dewlap will be less subject to predation and thus will be recaptured at higher rates than individuals with intact dewlaps. In a second experiment, we use clay models to test whether the presence of an extended dewlap increases the risk of avian predation. If detected, such costs are a potential mechanism for enforcing reliability in the green anole signaling system.

Methods

Animal Collection and Measurement

We captured anoles from a site near Augusta, GA, USA. In animals from this site, dewlap size is positively correlated with maximum bite force, even independent of body size (Henningson & Irschick, 2012). We collected animals by hand or by noose and transported them to the Savannah River Ecology Laboratory. There, we measured snout-vent length (SVL) to the nearest mm using digital calipers (Mitutoyo CD-8 CS, Aurora, IL, USA). We measured body mass to the nearest 0.01 g with a digital balance (Bettler AE163, Columbus, OH, USA). We measured dewlap size by holding each animal on a tabletop, extending its dewlap maximally with forceps, and photographing the extended dewlap with a ruler. We then measured dewlap area from these photographs using ImageJ (NID, Bethesda, MD, USA). We also measured maximum bite force performance following standard techniques (Herrel *et al.*, 1999). Briefly, males we induced to bite of the ends of custom cantilevered bite force plates connected to a force transducer (type 9023; Kistler Inc. Wintherthur, Switzerland). The transducer then sent a signal to a charge amplifier (type 5058a; Kistler Inc.), which displayed the maximum bite force. Animals bit five times in two sessions of either 2 or 3 bites per session, with at least one

hour of rest between sessions. We used the maximum force obtained as our measure of maximum bite force capacity.

Surgical Treatment

Males >50 mm SVL were alternately assigned to one of two groups: Disabled and Control. Disabled animals were surgically prevented from extending their dewlap following Crews (1975), Tokarz (2002), and Tokarz et al. (2003, 2005). We anesthetized each lizard with a subcutaneous injection of lidocaine (1 mg/kg) in the anterior and proximal region of the dewlap. We used surgical tape to hold the animal in place on a partially thawed chemical ice pack. We extended the dewlap and then made a small incision in the skin on the right side of the animal just posterior to the junction of the second ceratobranchials and the ceratohyals. We then exposed and severed the second ceratobranchial cartilages just distal to the ceratohyals (Figure 1; see Bels, 1990 for dewlap anatomy). We closed the incision with surgical glue and placed each animal individually into a holding cage overnight. Control group animals received identical treatments, except the cartilages were probed but left intact. Animals treated with this or similar dewlap manipulation procedures resume typical behaviors, including basking, feeding, and head-bobbing within hours (Crews, 1975; Tokarz, 2002; Henningsen & Irschick, 2012)

On the day following treatment, we marked the animals permanently and individually with a subcutaneous colored elastomer implants (Northwest Marine Technologies, Shaw Island, WA, USA) and then returned them to their exact place of

capture. We also marked these animals dorsally with a small dot of white paint so that we would not capture them again during the same collecting period.

Collection Dates and Recaptures

We initially collected animals and subjected them to treatment during three periods:

March 9, 2008 to March 20, 2008; September 11, 2008 to September 24, 2008; and April 30, 2009 to May 26, 2009. We continued to capture animals intermittently at the site from the end of the final period until September of 2011 for other research, but no further animals were treated for this experiment. We determined recapture success during these same periods and the subsequent work at the site until September 2011. If, during any of these periods, we caught an animal a second time, we counted it as a recapture event.

Clay Models

We created clay models of green anole lizards in three different states: one model had no dewlap, while a second set of models had a fully extended dewlap. Within the latter group, half of the models had a pink dewlap typical of *A. carolinensis*, while we gave the other half dewlaps the same green color as the body. We constructed models by making molds from preserved adult male green anoles, and creating plastic casts from these molds. We then covered the casts with a thin layer of modeling clay. We placed these models in our study site during a period between April and July 2010. Each model was attached to natural vegetation in the habitat with monofilament line in a position that appeared characteristic of the species. We returned after 72 hours at which time we examined the models for evidence of predation attempts. Following McMillan and

Irschick (2010), we determined whether models had been subjected to predation attempts by the presence or absence of triangular depressions in the model characteristic of attacks from bird predators.

Statistical Analyses

We used a *G*-test with Williams's correction (Sokal & Rohlf, 1995) to test whether animals with disabled dewlaps were recaptured at a significantly higher rate than those with intact dewlaps. We then used a generalized liner model to determine which traits significantly influenced probability of recapture. In this model, we used snout-vent length (SVL) as our proxy for body size. We also included measurements of mass, dewlap size before treatment, and maximum bite force capacity. Because these three traits are all positively correlated with SVL, we used size corrected measures. Namely, we used the residuals of each variable respectively regressed on SVL. Our model used a binomial error distribution with recapture (yes or no) as a response variable. From this model, we calculated odds-ratios for each variable. This statistic gives an estimate of how a change in one model parameter influences probability of recapture.

We used another *G*-test with Williams's correction to test whether our clay models with different dewlap conditions were subject to different rates of predation. We used R 2.15.1 (R Development Core Team, 2008) for all analyses.

Results

We used a total of 116 male green anoles. Though we generally alternated treatment, our final sample includes 61 animals with disabled dewlaps and 55 control animals.

We recaptured 14 of 61 individuals with disabled dewlaps and 12 of 55 control animals. The proportion of recaptures does not differ significantly between dewlap-disabled and control animals ($G = 0.021$, $p = 0.89$).

Neither original dewlap size, bite force, body mass (all size-corrected), nor dewlap treatment affected the probability of recapture. However, SVL was a significant factor in the generalized linear model. Our model predicts that for each 1 mm increase in SVL, the probability of recapture increase by approximately 16% (Table 1).

We recorded bird predation attempts on all three types of clay models (Table 2). There was a trend for models with pink dewlaps to be attacked more often than models with green dewlaps or models without dewlaps, it was not statistically significant ($G = 5.65$, $p = 0.059$).

Discussion

In Zahavi's original formulation of the Handicap Principle, the author described the risks incurred by a male songbird due to colorful plumage (Zahavi, 1975), and suggests the presence of the such conspicuous coloration proves the quality of the male because "it has already withstood the extra predation risk involved in its plumage" (Zahavi, 1975). Since Zahavi wrote that, a number of studies have provided direct evidence of predator-related costs (discussed above), and further theoretical developments have supported the idea that costs are necessary to enforce reliability (Grafen, 1990; Johnstone, 1997). Contrary our prediction, animals with intact dewlaps were recaptured as often as animals with disabled dewlaps. The presence of dewlaps on models, however, provided a hint of conflicting results. Among the models, there was a trend for pink dewlaps to be a target

of predators more often than models with green or no dewlaps. Overall, these results suggest that though predators may sometimes use conspicuous dewlaps to locate potential prey, other sources may impose costs on signalers, and male green anoles use other means to ameliorate the risk of displaying. Unlike the colorful signals in birds, fish, and stationary clay models, for example, the dewlaps of living anoles are displayed only intermittently and otherwise remain inconspicuous. Though dewlap display rates in green anoles can be as high as 100 per hour during the breeding season (Jenssen *et al.*, 1995), males can presumably modify display patterns when predators are observed. Perhaps paradoxically, green anoles also perform dewlap displays directed at predators, including human researchers (pers. obs.). Thus, if there are predation costs to territorial or courtship dewlap displays, there may also be benefits to the display when it is directed at predators.

Of course predators are not the only source that imposes costs on signals. Conspecific rivals can impart costs by punishing dishonest signalers (Rohwer, 1977; Searcy & Nowicki, 2005). Such receiver-dependent costs can occur when an individual with a dishonestly intense signal encounters a rival with an honest signal of equal intensity. If the encounter escalates to aggression, the dishonest individual may suffer defeat and even injury from the honest high-quality rival. Receiver-dependent costs are present in Harris's Sparrows, where males with artificially enlarged color badges become increased targets of aggression by males with naturally large signals (Rohwer, 1977). Costs can also result from displays that place the signaler in a position of vulnerability to retaliation, such as the soft-songs of some passerine birds (Hof & Hazlett, 2010). Hermit crabs with experimentally-increased vulnerability are less likely to escalate contests, suggesting vulnerability costs are real and dangerous (Laidre, 2007). In green anoles,

however, as male competitors increase in proximity during encounters dewlap displays are used less frequently (Decourcy & Jenssen, 1994; Jenssen *et al.*, 2000), making it unlikely that vulnerability costs are a major factor in enforcing reliability. Receiver-dependent costs may be present in green anoles, but previous work manipulating dewlap size to create dishonest signalers (in that case, modest signalers, i.e., having smaller dewlaps), showed no effect of dewlap size on the outcome of staged interactions between males (Henningsen & Irschick, 2012). That experiment reduced dewlap size to create modest signalers, but the alternate condition - creating boastful signalers with enlarged dewlaps - may be a more appropriate method to test for socially-enforced costs.

In addition to predation risk, there may be receiver-independent costs associated with the development or maintenance of a signal. Lailvaux *et al.* (2012) propose that reliability in the signaling system of green anoles stems from costs associated with developmental investment in bite force. Male green anoles fed a nutritionally-poor diet suffered no reduction in the size of dewlaps, but had lower bite forces than animals fed unrestricted diets (Lailvaux *et al.*, 2012). Developmental costs of the dewlap itself are thus unlikely to be a significant factor in enforcing reliability. Maintenance costs are another class of receiver-independent cost which are incurred after the development of the signal, but when the signal is not in use. One often-expected maintenance cost of signals is diminished locomotor performance (Oufiero & Garland, 2007). The dewlap however is often retracted and its relatively small size and low mass are unlikely to impede locomotion in any way. There is evidence that dewlap size is largest during the breeding season and decreases in size after this period (Irschick *et al.*, 2006), but whether this is an adaptive response to reduce costs is unknown.

In our statistical model, the only variable that significantly influenced probability of recapture was body size. Larger males were more likely to be recaptured in subsequent collecting periods. This pattern could be indicative of differential costs. Namely, large males may be better able to bear the cost of dewlap displays than smaller males. Differential costs are a major tenet of signaling theory (Getty, 1998; Kotiaho, 2001), but have been very difficult to demonstrate. We know of no other research demonstrating the presence of differential cost of a sexual signal. Our results here are suggestive that there may be predator-enforced costs to a dewlap signal, and large males are better able to withstand those costs.

Though the contexts in which *Anolis* lizards use their dewlaps are known, the purpose of the signal remains generally enigmatic. Unfortunately, we still do not know what costs, if any, are incurred via displaying the dewlap. These costs might accrue from multiple independent sources. Both predators and rival males could target large dewlap displays, for example. Considering that dewlap displays are used in multiple contexts (e.g., courtship, territorial displays, and displays to predators), these costs may be difficult to disentangle. Both predation intensity and male competition can vary greatly both between populations (McMillan & Irschick, 2010), and among species (Lailvaux & Irschick, 2007), particularly island and mainland groups (Losos, 2009). Analogous work in fiddler crabs has detected population level differences in reliability and receiver-dependent costs (Bywater & Wilson, 2012). Using these natural comparisons to examine how dewlap use and its effect differs may elucidate the benefits and costs related to the signals of *Anolis* lizards.

Acknowledgements

We thank B. Morris, R.Herman, B. DeGreggorio, and S. Reddington for assistance in the field. Thanks to T. Tuberville, W. Gibbons, and S. Lance at Savannah River Ecology lab for logistical assistance. Funding provided by National Science Foundation GRFP and DDIG #1011246, The American Philosophical Society, Society for Integrative and Comparative Biology, Animal Behavior Society, and the U. of Massachusetts Natural History Collection. All work was conducted with approved animal use protocols from University of Massachusetts (#28-10-01) and University of Georgia (#A2009-10092).

Table 2.1 Model parameters describing the probability of recapture.

	Estimate	S.E.	<i>z</i>	Odds Ratio (95% CI)
SVL	0.15	0.05	2.97*	1.16 (1.06-1.29)
Bite Force	0.17	0.19	0.93	1.19 (0.83-1.75)
Initial Dewlap Size	0.01	0.01	1.62	1.01 (0.99-1.02)
Mass	-0.60	0.43	-1.41	0.55 (0.22-1.24)
Dewlap Treatment	-0.39	0.55	-0.72	0.68 (0.23-1.96)

* $p < 0.01$

Table 2.2 The number of clay models showing evidence of predation attempts.

	Attacked	Not Attacked
Pink Dewlap	8	27
Green Dewlap	2	33
No Dewlap	2	30

Figure 2.1 An X-ray of *Anolis carolinensis* head and dewlap anatomy. The thick white line distal to the junction of the basihyal and ceratobranchial 1 shows the location where the second ceratobranchial 2 cartilages were severed. Image courtesy Beth Brainerd.

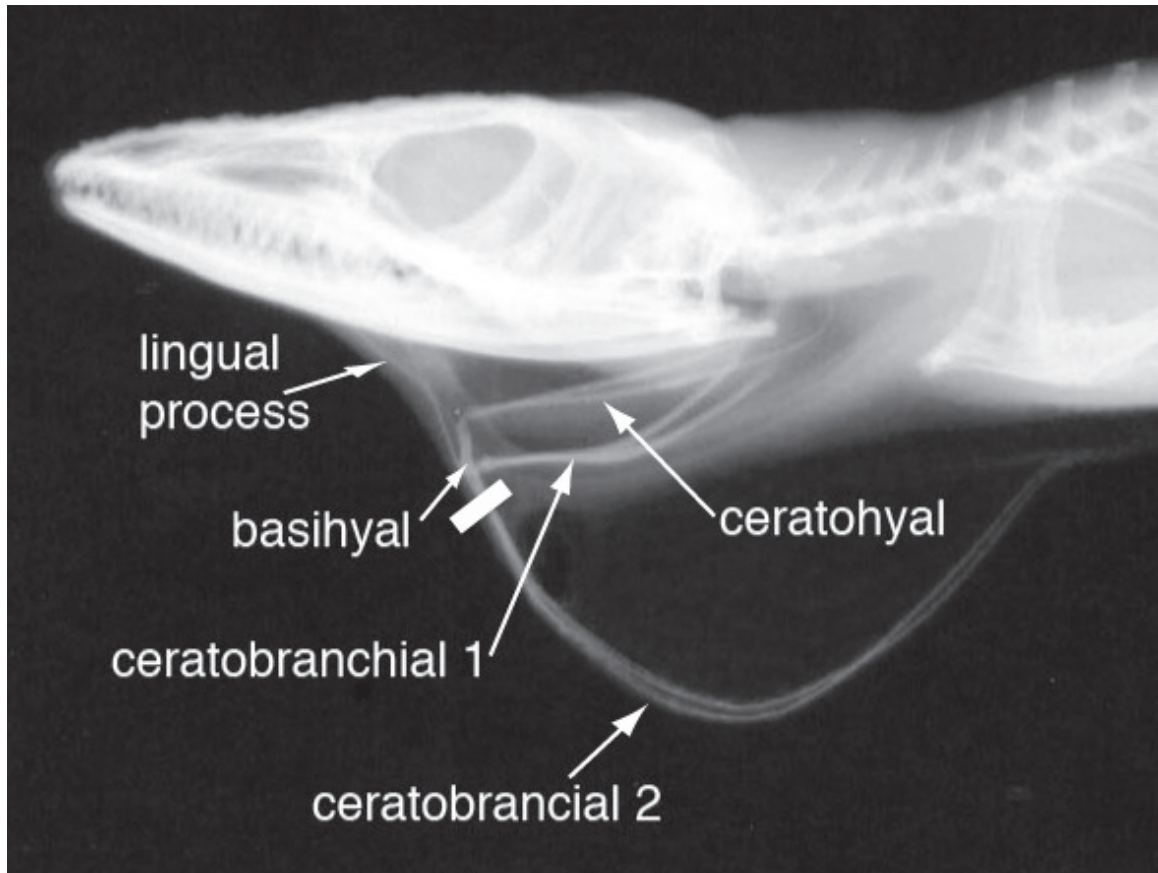
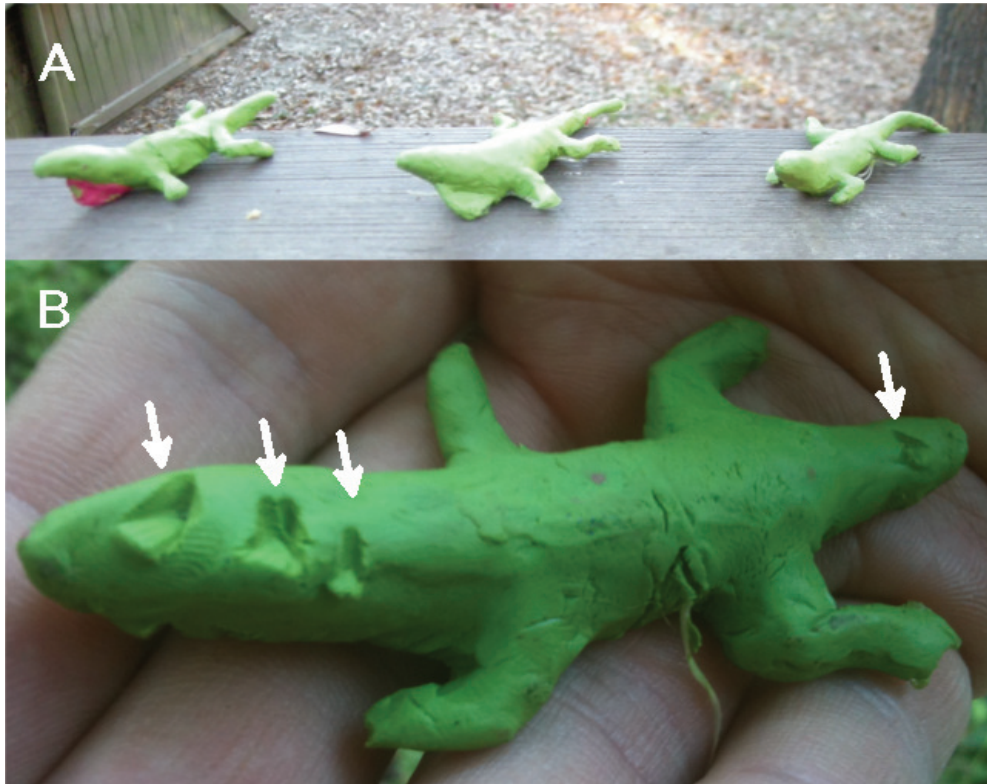


Figure 2.2 (A) Representatives of the three types of clay models used. From left to right: Pink Dewlap, Green Dewlap, No Dewlap. (B) A model with typical marks from a predation attempt



CHAPTER 3

ARE SEASONAL CHANGES IN A PERFORMANCE TRAIT AND ITS RELIABLE SIGNAL MEDIATED BY TESTOSTERONE? EXPERIMENTS UNDER BREEDING AND NON-BREEDING CONDITIONS

Many animal species possess elaborate secondary sexual traits, and these traits are often subject to seasonal changes. From an ultimate perspective, the high cost of developing secondary sexual traits during a particular season suggests that these traits have otherwise adaptive functions that offset their production costs. (Andersson, 1994; Adkins-Regan, 2005; Searcy & Nowicki, 2005). At a proximate level, seasonal changes in secondary sexual traits in vertebrates often occur in response to androgens, particularly testosterone (Marler & Moore, 1988; Wingfield *et al.*, 1990; Andersson, 1994; Ketterson *et al.*, 2001; Oliveira, 2004; Adkins-Regan, 2005; Hau, 2007). Given the need to coordinate timing between readying gonads for reproduction and secondary sexual characteristics, it is logical that steroid hormones mediate changes in both (eg, Goeritz *et al.*, 2003; reviewed in Adkins-Regan, 2005). For example, in song sparrows (*Melospiza melodia*) seasonal increases in singing behavior and growth of associated brain nuclei positively correlate with seasonal increases in testosterone levels (Smith *et al.*, 1997).

However, androgens vary greatly in their effects on traits temporally associated with breeding. Not all secondary sexual traits that undergo seasonal changes are under the influence of testosterone. Experiments with canaries (*Serinus canaria*), a model organism for seasonal changes in the brain structures controlling singing behavior, showed the changes in these structures occur independent of androgen levels (Brown, Johnson & Bottjer, 1993). Even within individuals, some secondary sexual structures change in

response to testosterone, but others are insensitive. For example, (1998) showed that forelimb musculature in Leopard Frogs (*Rana pipiens*), which is sexually dimorphic and used by males during copulation, differed in responsiveness to treatment with testosterone: some muscles showed sharp increases in dry mass while others did not change. Importantly, hormonally caused responses can differ between seasons. For example, in European Stonechats (*Saxicola torquata*), androgens and estrogens stimulate aggressive behavior, but only during the breeding season (Canoine & Gwinner, 2002). In contrast, in Song Sparrows (*Melospiza melodia*) steroid hormones can stimulate aggressive territorial behavior during the breeding season when testosterone concentrations are high, and during the non-breeding season when testosterone levels are low (Soma & Wingfield, 2001).

Because of the variation in responsiveness to hormones, one way of explicitly testing the role of a hormone in seasonally changing traits is via experimental manipulation. Changing hormone levels at one or more times and observing the effects can elucidate the relationship between a hormone and the traits in question, including seasonally-dependent changes in sensitivity. In this paper, we describe two experiments that test, in male green anole lizards, the relationship between testosterone and three phenotypic traits: dewlap, bite force performance, and maximum sprint speed.

As is the case with many animals in temperate climates, the life history and reproductive biology of green anole lizards (*Anolis carolinensis*) are influenced by seasonal weather patterns. Green anoles are geographically widespread across the southeastern United States such that the exact timing of seasonal patterns may differ between populations. A well-studied population located in Georgia breeds from May

through July (Jenssen *et al.*, 1995; Jenssen, Lovern & Congdon, 2001). At the onset of the breeding season in this population, adult male anoles experience a rapid spike in circulating testosterone levels (Jenssen *et al.*, 2001). When the breeding season concludes, activity levels, territoriality, steroid hormone levels and gonad size all decrease (Jenssen *et al.*, 1995, 2001). Concomitant with this pattern, two other relevant traits undergo seasonal changes, namely dewlap size and maximum bite force (Irschick *et al.*, 2006). Like most male *Anolis* lizards, display their dewlaps during social interactions, including aggressive encounters between males (Greenberg & Noble, 1944; Jenssen *et al.*, 1995). When dewlap signaling fails to resolve an aggressive conflict, encounters between males may escalate to physical altercations that result in opponents biting one another (McMann, 1993; Jenssen, Decourcy & Congdon, 2005), and prior laboratory studies have shown a link in green anoles between maximum bite force and dominance (Lailvaux *et al.*, 2004; Henningsen & Irschick, 2012).

In addition to how they change seasonally, dewlap size and bite force performance are of interest because of the relationship between them. During the breeding season, these traits exhibit a size-independent, positive correlation, suggesting that dewlap size is a reliable signal of bite force capacity (Lailvaux *et al.*, 2004; Vanhooydonck *et al.*, 2005a; Henningsen & Irschick, 2012). Because there are no known mechanical links between these traits that would explain their correlation, dewlap size can be considered a conventional signal of bite force capacity. To gain insight into the proximate mechanism that regulates the relationship between the traits in this reliable signaling system, we experimentally manipulated testosterone levels in green anole lizards to test its effects on seasonal changes in dewlap size and bite force capacity.

Another performance trait important in the resolution of aggressive interactions, between male lizards, in addition to bite force, is locomotor capacity (Garland, Hankins & Huey, 1990; Robson & Miles, 2000; Lailvaux *et al.*, 2004; Husak *et al.*, 2006; but see Perry *et al.*, 2004). Better jumping performance, endurance, and sprint speed have all been implicated as factors affecting dominance in lizards. Furthermore, testosterone is known to increase muscle mass, aerobic performance, and endurance in some species (Husak & Irschick, 2009). Therefore, in one experiment (see below) we measured a locomotor trait, sprint speed, to determine whether this performance trait changes seasonally and, if so, whether the change relates to levels of testosterone. Finally, we explore one potential morphological mechanism that could effect a change in dewlap size.

We used the following experimental design: We compared three groups (1) gonadectomized males given exogenous testosterone, (2) gonadectomized males given blank implants and (3) control males that received sham surgeries and blank implants. We conducted two different experiments, each in a different time period: within the breeding season (May-June), and during the non-breeding season (July-August). Using this design, we addressed these questions: First, does testosterone cause seasonal changes in dewlap size, bite force performance, and sprint speed? Do these responses vary between breeding and non-breeding seasons? Finally, we examine the relationship between second ceratobranchial length and dewlap size as one potential mechanistic explanation to explain how dewlap size may change.

Methods

Experiment 1: Non-breeding season

We captured sexually mature male green anoles by noose or hand from a population in Destrehan, LA, USA between 27 April and 1 May 2007. We collected animals during the breeding season, but held them in isolation in the lab until after the end of the breeding season (see below). Male anoles from this population are known to have a bimodal size distribution, which may indicate that animals at different life stages experience different selection pressures (Lailvaux *et al.*, 2004; Bloch & Irschick, 2006; Irschick *et al.*, 2006), so we used only “heavyweight” individuals (>64 mm SVL). We transported animals to the laboratory in Amherst, MA, USA, where they were housed individually in 13 L (32 x 20 x 20 cm) plastic aquaria. Incandescent light bulbs provided light and heat on a 12 h light : 12 h dark cycle. The room was maintained at approximately 25° C, and lizards had access to higher temperatures provided by overhead heat lamps (60-W). We used constant conditions to replicate the conditions used by Irschick *et al.* (2006), in which seasonal changes in dewlap size and bite force performance occurred. The sides of the aquaria were covered such that animals could not see one another. Paper towels were used as substrate for ease of cleaning and each animal was provided with a perch of natural wood. The sides of the aquaria were misted with water daily and crickets were provided three times weekly. To remove any possible position effect, cages were repositioned in a random order each week after cleaning. We measured performance and morphology on 3 July 2007. We performed surgeries on 4 July 2007. We measured morphology and performance again on 2 August 2007, 30 days after surgery. Sample sizes for both experiments are in Table 1.

To explore one potential mechanism for variation in dewlap size, we measured the length of the second ceratobranchial cartilages. These paired cartilaginous rods form the outer margin of the dewlap, and their protraction causes the dewlap to be extended (Bels, 1990). After the animals were euthanized, we dissected out the second ceratobranchials. Then we stretched them out and placed them onto a table adjacent to a ruler and photographed them from above. We then used ImageJ to measure the length of the cartilages. Finally, we performed an ordinary least squares regression of dewlap size on cartilage length.

Experiment 2: Breeding Season

Animals for the breeding season experiment were collected at the Augusta Canal in Augusta, GA USA. Animals from this population have also been objects of study (e.g., Jenssen *et al.*, 1995; Lovern, McNabb & Jenssen, 2001; Orrell *et al.*, 2004). This population does not show the bimodal size distribution mentioned above (Henningsson & Irschick, 2012), so we used adult males that fall in both size classes defined in Lailvaux *et al.* (2004; 54.9 - 67.5 mm SVL). Animals were collected by noose or hand and transported to the Savannah River Ecology Lab near Aiken, SC, USA, where they were housed individually in conditions identical to the non-breeding experiment with the following exceptions. First, ambient temperatures in the room where the animal were housed averaged 32°C during the day, and 26°C at night. Next, overhead incandescent lights were on a cycle that matched the natural photoperiod and were adjusted to match the changes in day length every two weeks. The higher temperatures and natural photoperiod were chosen to simulate natural conditions and allow the animals to maintain

reproductive status. We collected these animals during March 25-April 5, 2010. Initial measurements of morphology and performance were done on April 08, 2010. We performed surgeries on April 09 and 10. We measured morphology and performance again (see below) on May 19, 38 days after the conclusion of surgery.

Surgeries and Implants

Surgical techniques and implant preparation were identical in the two experiments, with the exception of the differences noted below. We randomly assigned anoles to one of three treatment groups: (1) castrated with testosterone implant (TEST); (2) castrated with blank implant (CAST); (3) a control group given sham surgeries (CON). Castration methods were identical in the TEST and CAST groups and were performed as follows. In the non-breeding experiment, animals were anesthetized with an intraperitoneal injection of lidocaine and then immobilized by placing them on crushed ice, where surgeries were performed. In the breeding experiment, animals were anesthetized with an intramuscular injection of meloxicam and induction with isflurane. A small (8-10mm) incision was made ventrally. We then used sterile silk suture thread to ligate the spermatic cord proximal to a testis. The testis was then excised and the spermatic cord cauterized to destroy any remaining gonadal tissue. We repeated this procedure for the contralateral testis using the same incision. We then closed the incision with tissue adhesive (VetBond; 3M, St. Paul, MN, U.S.A.) and allowed the animals to recover. For the CON groups, we performed sham surgeries by anesthetizing the animal, making an identical incision, and gently prodding the testes without removing them.

We prepared implants following Lovern et al (2004). Crystalline testosterone propionate (Steraloids, Inc., Newport, RI) was mixed thoroughly in medical grade silicon sealant (Type A, Dow Corning) and placed in a 1-ml syringe. We then expelled a straight line of the mixture onto wax paper and allowed it to dry overnight. Implants were cut into cylinders 2 mm long and 2 mm in diameter, with the goal of having approximately 0.7 mg of testosterone per implant to reach a circulating plasma testosterone concentration of 30-40 ng/mL, the upper limit of natural variation in free-ranging adult males (Lovern *et al.*, 2004; Husak *et al.*, 2007). Blank implants were made in the same way, but lacked testosterone propionate.

Hormone Sampling and Assays

We collected blood for validation of castrations and testosterone-implants 4-5 weeks after surgical treatments and implants. Immediately after capture from the aquaria we collected whole blood from the suborbital sinus with a heparinized microhematocrit capillary tube. Blood was collected from all animals within a one-hour period to minimize diel variation in circulating hormone concentrations. Once a sample was collected it was transferred to a 0.75-ml microcentrifuge tube and placed on ice until the plasma fraction (mean volume \pm SEM = 11.4 ± 1.44 μ l) was separated by centrifugation and stored at -20° C until assays were conducted.

Concentration of testosterone was measured by standard radioimmunoassay (RIA) techniques following extraction and chromatographic separation (Wingfield & Farner, 1975). Since the details have been published elsewhere (Husak *et al.*, 2007) we only briefly describe techniques. We equilibrated each sample overnight with 2,000 cpm of

tritiated steroid to determine individual extraction efficiency. Each sample was extracted twice with 4 ml of diethyl ether with the ether phase removed and dried in a water bath, under a stream of nitrogen gas, and resuspended in 10% ethyl acetate in isooctane. To remove neutral lipids and to isolate 5 α -dihydrotestosterone (DHT) and testosterone, all samples were transferred to diatomaceous earth (Celite, Sigma) columns for chromatographic separation, after which only the testosterone fraction was retained for analysis. We then dried samples in a 40° C water bath under nitrogen gas, resuspended them in 600 μ l phosphate buffered saline, and maintained them overnight at 4° C. Individual extraction efficiency (mean recovery was 69%) was determined from 100 μ l of the sample while two duplicates of 200 μ l each of the sample were allocated for the assay. Serial dilutions for the standard curves were performed in triplicate (range of curve: 500 – 1 pg). All samples were then incubated overnight with 100 μ l of testosterone antiserum (WLI-T-3003S, Fitzgerald Industries, Concord, MA) and 100 μ l of tritiated steroid (10,000 cpm). Unbound steroid was separated using dextran-coated charcoal and the bound steroid decanted into scintillation vials. Samples were then counted on a liquid scintillation counter and final concentrations corrected for individual extraction efficiency. The average intra-assay coefficient of variation (CV) from six standards was 4% for both the non-breeding experiment and breeding experiments.

Morphology and Performance

In both experiments, we measured the following traits when the animals were brought to the laboratory. We measured snout-vent length (SVL) to the nearest 0.1 mm using digital calipers and mass to the nearest 0.01 g using a digital balance. We placed each animal on

its left side on a tabletop and used forceps to gently and fully extend the dewlap. We photographed each animal in this position with a scale bar and used these digital photographs to calculate the area of the extended dewlap (hereafter, dewlap size). One person (JPH) performed all measurements. This method provides reliable and repeatable results for measuring dewlap size (Vanhooydonck *et al.*, 2005a).

We measured maximum bite force following Herrell *et al.* (2001). We induced lizards to bite on padded metal plates connected to a force transducer (type 9023, Kistler, Inc., Wintherthur, Switzerland) connected to a charge amplifier (type 5058a, Kistler, Inc.). We acquired five bites from each lizard in two sessions (two or three bites per session) with at least 30 minutes of rest between sessions. We used the maximum of these five bites as our measure of maximum bite force performance. We measured bite force for all animals in the lab at an ambient temperature of approximately 24° C.

Sprint Speed

In the breeding experiment, we measured maximum sprinting speed for each lizard at two periods. We used a custom-built 2-meter racetrack set at a 45° angle. The track surface was a 4 cm diameter wooden dowel covered in a thin layer of cork. Lizards were placed in an incubator at 30° C for one hour prior to running. Lizards ran from the bottom of the track toward a patch of artificial plants at the top. We motivated lizards to run by chasing them with our hands without touching them. As a lizard ran, it passed a series of five pairs of evenly spaced photogates that recorded the time interval between sensors on a connected computer. Each run generated a speed for up to 7 intervals, and we used the fastest of these as our measure of maximum sprint speed in subsequent analyses.

Following Losos et al. (2002), we subjectively described each run as either ‘good’ or ‘bad’ based on our judgment as to whether the lizard was moving near maximum speed. In subsequent analyses, we excluded animals that did not have a ‘good’ run (N=2).

Statistical Analyses

We performed a one-way ANOVA to test for differences in plasma testosterone among treatment groups at the conclusion of each experiment. We used repeated-measures ANOVA to test for significant differences among treatments in changes in the size of the dewlap and maximum bite force. We used the same method to test for differences in sprint speed in the breeding experiment. We analyzed all data with R v2.15 (R Development Core Team, 2008).

Animal Use Protocols

All work was done in accordance with protocols OGCA #106-1749 from the University of Massachusetts Amherst and A2010-2-018 from the University of Georgia Institutional Animal Care and Use Committees.

Results

Experiment 1: Non-breeding Season

Treatments differed significantly ($F_{2,18} = 25.8$, $P < 0.0001$) in plasma testosterone concentrations at the conclusion of the experiment, with the TEST group having the highest levels of testosterone. (Figure 1A). A Tukey-Kramer’s HSD test showed that both the approximately 10-fold increase between the TEST and CAST groups ($p < 0.001$) and

the approximately 5-fold increase between the TEST and CON groups ($p < 0.001$) were statistically significant. There was no significant difference between the CAST and CONT groups ($p > 0.9$). The TEST group had a mean plasma testosterone concentration of 24.2 ng/ml, which is within the normal range of heavyweight males during the breeding season (Lovern *et al.*, 2004; Husak *et al.*, 2007).

Both mass and SVL increased significantly with time (Table 1), but neither differed significantly as a result of treatment or time x treatment interactions (Table 2, Figure 2B). There were no significant treatment, time, or treatment x time effects for dewlap size (Table 2, Figure 2B). Dewlap size in the TEST and CONT group was virtually unchanged over time, while dewlap size in the CAST group decreased non-significantly by approximately 5%. Bite force decreased significantly over time in all groups by an average of approximately 20%. However, neither treatment nor treatment x time interaction significantly affected bite force.

Because there were no significant differences in dewlap size among groups, and the lengths of the second ceratobranchial cartilages (CBII) did not differ significantly among groups (One-way ANOVA; $F_{2,18} = 2.44$, $P = 0.12$), we could not explicitly test whether differences in dewlap size are the results of changes in CBII length. To test whether individual variation in CBII length explains individual variation in dewlap size, we regressed dewlap area on CBII length and found no significant relationship ($F_{1,19} = 0.053$, $r^2 = 0.027$, $P = 0.48$).

Experiment 2: Breeding Season

There was a significant difference in plasma testosterone between treatment groups ($F_{2,12} = 14.84, p < 0.001$; Figure 1). The approximately 10-fold difference between the mean of the TEST group and that of the CAST groups (Tukey's HSD test; $p < 0.001$) and the 5-fold difference between the mean of the TEST and that of the CONT group ($p = 0.003$) were statistically significant. There was no statistically significant difference between the CONT and the CAST group, despite the CONT group having a mean 8-fold higher than the mean of the CAST groups.

During the breeding season experiment, both mass and SVL increased with time, but neither was significantly influenced by hormone treatment or time x treatment interactions (Table 2, Figure 2A). There was no significant effect of time, treatment, or time x treatment interaction on dewlap size, which remained relatively constant in all groups. Bite force both increased over time in all three groups in the range of 13-18%, but the changes were not dependent on hormone treatment (Table 3). In contrast, there was a significant interaction effect of time x treatment on maximum sprint speed performance. This effect seems to be driven by an approximately 75% increase in maximum sprint speed among the TEST group. The CONT group showed a moderate increase in sprint speed over this period, while the CAST group speed did not change.

Discussion

When secondary sexual traits change seasonally and concomitantly, a common regulatory mechanism may control and synchronize the changes. In our experiments, bite force and sprint speed changed significantly over time, while dewlap size did not. Despite concurrent changes in sprint speed and bite force, the traits differed in their response to

testosterone or castration. During both experiments (breeding and non-breeding seasons), bite force increased significantly over time, but these changes were independent of hormone treatment. In contrast, sprint speed showed a significant time by treatment interaction, with the high-testosterone group showing a significant initial increase in sprint speed. Taken together, our results do not support the role of testosterone as a regulatory mechanism of the reliable signaling system of dewlap size and bite force, but show that other traits (i.e., sprint speed) are responsive to these seasonal changes.

The effects of hormones are often classified according to a temporal continuum: Organizational effects take place during development, while activational effects result in short-term changes such as the seasonal patterns we investigate here. Testosterone has an organizational effect in the dewlap of green anoles (Lovern *et al.*, 2004). Exogenous testosterone given to juveniles during development increased two components of dewlap morphology: the cross-sectional area of the muscles that control dewlap extension (ceratohyals), and the length of the second ceratobranchial cartilages (Lovern *et al.*, 2004; see below). By contrast, our results show no obvious influence of exogenous testosterone on dewlap size. However, a separate castration and hormone replacement experiment in brown anoles (*A. sagrei*) showed that under breeding conditions, dewlap size decreased in castrated males relative to males with higher levels of testosterone (Cox *et al.*, 2009). Overall, these disparate findings suggest different effects and targets of testosterone both among different regions of the dewlap, and among different species.

The specific anatomical components that cause seasonal changes in dewlap size remain unknown. In green anoles, the second ceratobranchials and their associated musculature (the ceratohyals) both possess androgen receptors (Holmes & Wade, 2005).

However, in contrast to its effect on copulatory tissues, testosterone does not increase the number of receptors in the dewlap (Holmes & Wade, 2005). To the best of our knowledge, it is unknown whether the density of androgen receptors in the ceratobranchial cartilages changes seasonally. Though the second ceratobranchial cartilages show male-biased sexual size dimorphism in green anoles and this dimorphism is testosterone-dependent (Lovern *et al.*, 2004), our results show no correlation between the length of the cartilages and dewlap size among males. Overall, there seems to be no link between the size of main cartilaginous elements that control dewlap extension, testosterone, and dewlap size. One potential area for future work is on other major anatomical components that may determine dewlap size, such as the skin of the dewlap. Seasonal changes in skin elasticity may influence the degree to which the ceratobranchials can be adducted during a dewlap display, thus determining the effective size of the dewlap.

A modest number of studies have considered the effect of seasonality on measures of whole-organism performance. Some of the first research on seasonal changes in performance showed that endurance capacity in some lizards increased during the breeding season (John-Alder, 1984; Garland & Else, 1987). And though other work has demonstrated seasonal changes in performance (Rimmer, Saunders & Paim, 1985), many experiments yield equivocal results. Two separate studies in other lizards failed to find any relationship between testosterone levels and sprinting performance (O'Connor *et al.*, 2011) or bite force performance (Huyghe *et al.*, 2010). Male brown anole lizards (*A. sagrei*) given exogenous testosterone had higher bite forces than their castrated counterparts, but this difference may have been an artifact of increased growth in

testosterone-treated individuals (Cox *et al.*, 2009). Taken together, the results of previous experiments suggest that there is no one pattern that describes how testosterone concentration changes performance traits.

Bite force performance has been well studied in lizards. Male green anoles from a population near New Orleans, LA, USA show marked seasonal differences in bite force performance between spring and winter (Irschick *et al.*, 2006). However, this pattern is largely due to a reduction in overall variation in bite force between seasons, with hard-biting green anoles changing very little, and weak-biting green anoles changing a great deal by increasing their bite forces (Irschick *et al.*, 2006). The observed pattern is somewhat surprising because males are most likely to fight and bite during the breeding season (Jenssen *et al.*, 1995), when high bite forces are an advantage (Lailvaux *et al.*, 2004; Henningsen & Irschick, 2012). One possible explanation for the discrepancy between previous results and those in our study is geographic variation in social behavior and other life-history traits, which has not been examined widely in green anoles (but see Lovern *et al.*, 1999; Edwards & Lailvaux, 2012), but a study comparing two Louisiana populations that exist within about 20 km of one another yet occur in divergent habitats describes divergence in social behavior (Bloch & Irschick, 2006). We note again that we used animals from different populations in each experiment. Using animals of unknown origin (i.e., from commercial dealers) is relatively common in previous hormone work on green anoles, and we recognize the limitations of such methods. Thus, here we avoid direct statistical comparisons of animals from different populations and focus instead on the outcomes within each experiment.

Hormones often have pleiotropic effects, but different phenotypic components within an individual can evolve responses to hormones independently (Hau, 2007), and our data are consistent with previous work in green anoles supporting the “evolutionary potential hypotheses,” which outlines the lability of individual traits and their response to endocrine signals (reviewed in Hau, 2007). In contrast with our results for bite force, there was a significant effect of hormone treatment on sprint speed. Though the average maximum speed increased in each treatment group, the approximately 75% increase in sprint speed in the control group was much greater than the approximately 25% and 16% increases in the control and castrated groups, respectively. This result is consistent with both the popular notion and the empirical evidence that testosterone can increase performance in human athletes by stimulating muscle growth (Husak & Irschick, 2009). Our sprint speed results contrast with testosterone manipulations of brown anoles (*A. sagrei*) by Cox et al. (2009), wherein testosterone treatment had no effect on sprint speed. In our experiment, the significant interaction of treatment by time suggests that testosterone may be stimulating muscle growth or other physiological processes that result in increased sprinting speed. The disparate patterns observed in brown (Cox *et al.*, 2009) and green anoles (this study) suggest that testosterone may have evolved different regulatory roles within the genus *Anolis*.

Overall, our results corroborate previous work in green anoles that showed the response to testosterone varies in different parts of the individual (Lovern *et al.*, 2004; Holmes & Wade, 2005; Holmes, Bartrem & Wade, 2007; Husak *et al.*, 2007). The specific mechanisms responsible for the variation in responsiveness to testosterone in green anoles remain unknown, and the topic is ripe for further research.

Reliable, conventional signals require coordination between the signal and the trait being advertised for reliability to be maintained, particularly when these traits change seasonally. Despite the relationship between dewlap size and bite force in male green anoles and the fact that both traits are changing between breeding and non-breeding periods, changes in neither trait seem to be mediated by testosterone. Future work determining how changes in these two traits are regulated could provide fascinating insight into the evolution and maintenance of reliable signaling system.

Acknowledgements

We thank E. Brainerd for helpful discussion regarding hyoid anatomy and experimental design. We also thank J. Bailey, B. DeGregorio, R. Herman, T. Tuberville, P. Widder, L. White, J. Wood, and for their generous assistance and the Extac Corporation for access to the Louisiana field site. We thank the Behavior and Morphology group at UMass Amherst for helpful comments on the manuscript. Funding provided by NSF Graduate Research Fellowship to JPH, NSF DDIG IOS-1011246 to DJI and JPH, NSF IOB-0421917 to DJI and NSF IOS-0545735 to ITM.

Table 3.1 Descriptive statistics of the data measured in each experiment

a) Non-breeding season

	Dewlap Area (mm ²)	Bite Force (N)
TEST (N=10)		
July	227.5 ± 8.3	13.0 ± 0.81
August	231.7 ± 6.4	10.3 ± 0.49
CAST (N=6)		
July	238.3 ± 7.1	12.4 ± 1.21
August	237.3 ± 4.4	10.4 ± 0.59
CON (N=9)		
July	242.7 ± 8.5	12.4 ± 0.41
August	229.1 ± 6.6	9.6 ± 0.37

b) Breeding season

			Sprint Speed (cm/s)
TEST (N=5)			
April	231.50 ± 8.4	8.23 ± 0.21	53.55 ± 6.3
May	227.03 ± 15.1	9.28 ± 0.29	93.73 ± 13.4
CAST (N=6)			
April	225.12 ± 12.4	8.52 ± 0.40	50.05 ± 6.6
May	218.35 ± 5.5	9.88 ± 0.60	58.28 ± 7.6
CON (N=5)			
April	243.13 ± 11.3	8.41 ± 0.34	58.74 ± 7.7
May	244.62 ± 11.1	9.96 ± 0.43	72.10 ± 3.5

Table 3.2 Non-breeding season experiment

Trait	F (d.f.)	p
SVL		
Time	6.667 (1,22)	0.0169
Hormone Treatment	0.364 (2,22)	0.6992
Time x Treatment	1.867 (2,22)	0.1783
Mass		
Time	29.7482 (1,22)	< 0.0001
Hormone Treatment	0.1315 (2,22)	0.8774
Time x Treatment	2.1822 (2,22)	0.1366
Bite Force		
Time	8.2182 (1,22)	0.0090
Hormone Treatment	2.9729 (2,22)	0.0720
Time x Treatment	0.1310 (2,22)	0.8779
Dewlap Size		
Time	0.8636 (1,22)	0.3628
Hormone Treatment	0.3852 (2,22)	0.6848
Time x Treatment	1.7431 (2,22)	0.1983

Table 3.3 Breeding season experiment

Trait	F (d.f.)	p
SVL		
Time	12.536 (1,14)	0.003
Hormone Treatment	0.553 (2,14)	0.727
Time x Treatment	1.165 (2,14)	0.341
Mass		
Time	12.97 (1,14)	0.003
Hormone Treatment	0.55 (2,14)	0.587
Time x Treatment	0.480 (2,14)	0.629
Bite Force		
Time	40.839 (2,14)	< 0.0001
Hormone Treatment	0.240 (2,14)	0.790
Time x Treatment	0.545 (2,14)	0.592
Dewlap Size		
Time	0.205(1,13)	0.658
Hormone Treatment	2.065 (2,13)	0.166
Time x Treatment	0.035 (2,13)	0.966
Sprint Speed		
Time	16.151 (2,24)	0.002
Hormone Treatment	1.949 (2,12)	0.185
Time x Treatment	4.177 (2,12)	0.042

Figure 3.1: Plasma testosterone levels four weeks after treatment for (a) the breeding season experiment, (b) the non-breeding season experiment. Whiskers are Standard Errors of the Mean.

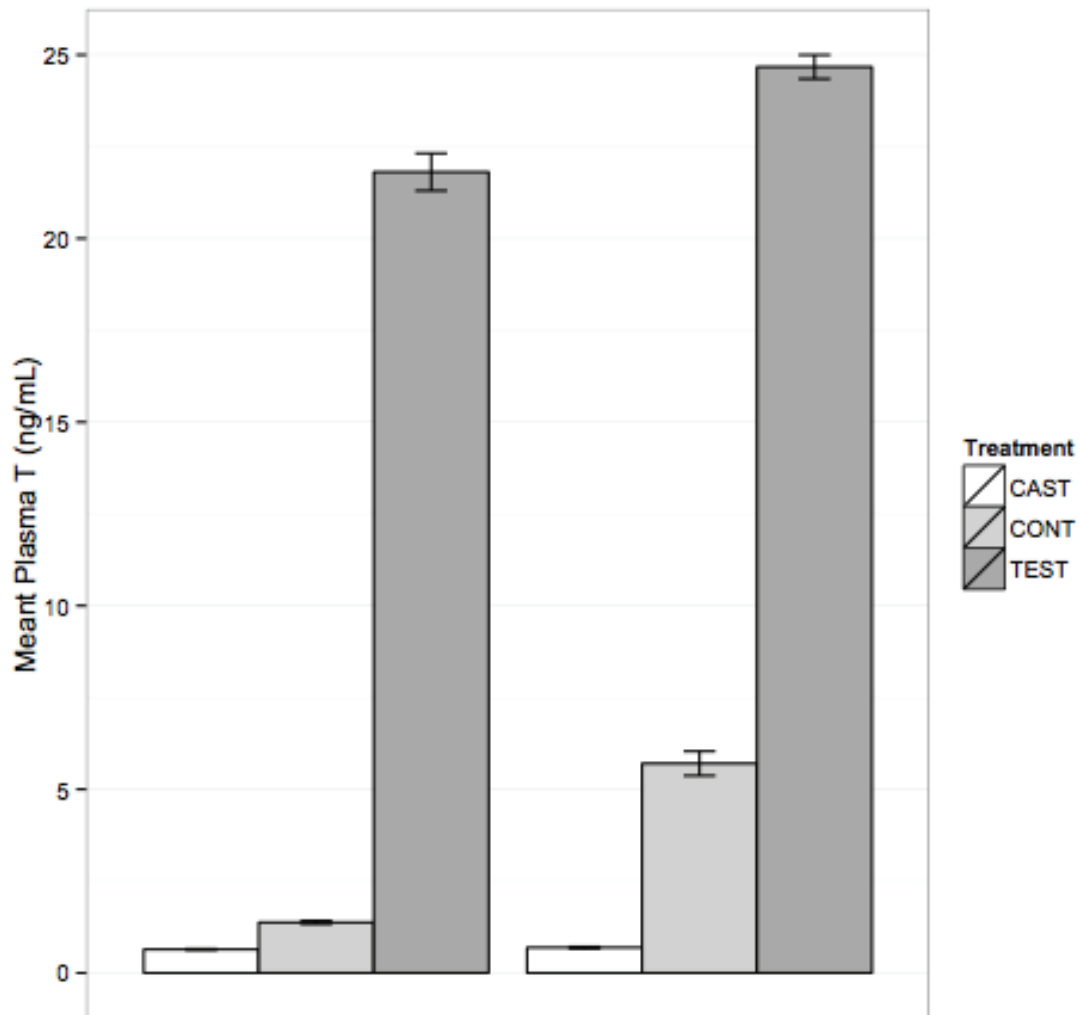


Figure 3.2: Box and whisker plots of four variables for (a) the breeding season experiment and (b) the non-breeding season experiment. Central black lines show the median, the edges of the boxes are the 25th and 75th percentiles, and the ends of the lines are the minima or maxima within 1.5 times the interquartile range. Values outside this range are represented by individual points.

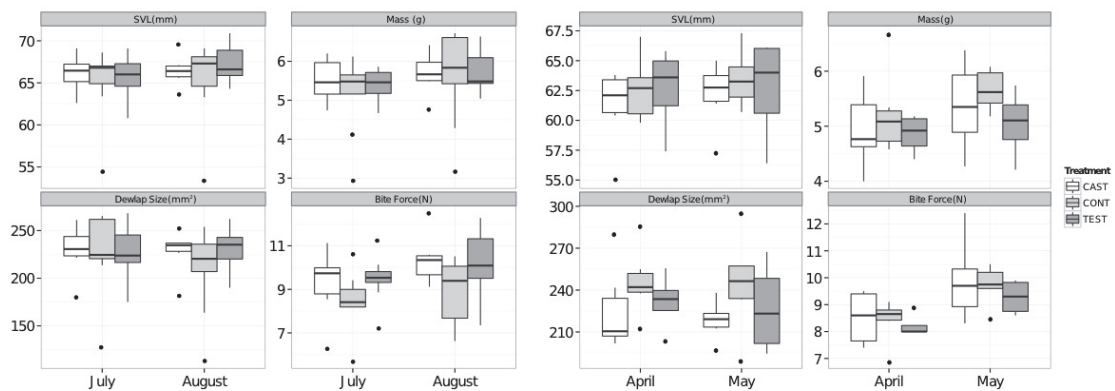
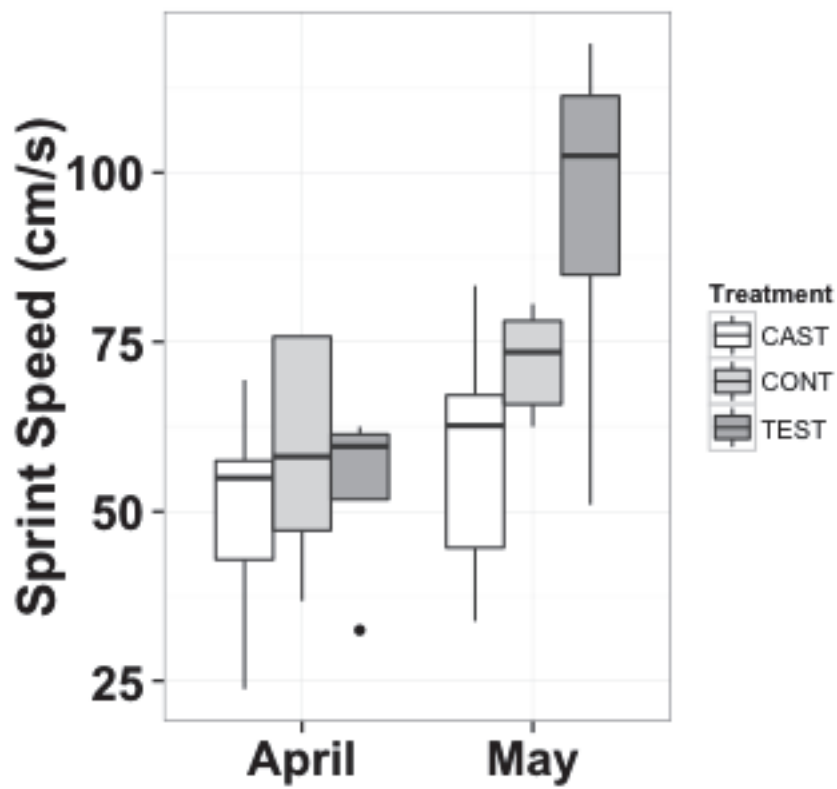


Figure 3.3: Box and whisker plot of maximum sprint speed during the breeding season experiment. Boxplot elements are the same as Figure 3.2.



BIBLIOGRAPHY

- Adams, E.S. & Caldwell, R.L. (1990). Deceptive communication in asymmetric fights of the stomatopod crustacean *Gonodactylus bredini*. *Anim. Behav.* **39**, 706–716.
- Adkins-Regan, E. (2005). *Hormones and Animal Social Behavior*. , Monographs in Behavior and Ecology. Princeton, NJ: Princeton University Press.
- Andersson, M. (1994). *Sexual Selection*. , Monographs in Behavior and Ecology. Princeton, New Jersey: Princeton University Press.
- Andrews, R.M. (1985). Mate Choice by Females of the Lizard, *Anolis carolinensis*. *J. Herpetol.* **19**, 284–289.
- Arnold, S.J. (1983). Morphology, Performance and Fitness. *Am. Zool.* **23**, 347–361.
- Arnott, G. & Elwood, R.W. (2008). Information gathering and decision making about resource value in animal contests. *Anim. Behav.* **76**, 529–542.
- Arnott, G. & Elwood, R.W. (2009). Assessment of fighting ability in animal contests. *Anim. Behav.* **77**, 991–1004.
- Bels, V.L. (1990). The Mechanism of Dewlap Extension in *Anolis-Carolinensis* (Reptila, Iguanidae) with Histological Analysis of the Hyoid Apparatus. *J. Morphol.* **206**, 225–244.
- Bloch, N. & Irschick, D.J. (2006). An Analysis of Inter-Population Divergence in Visual Display Behavior of the Green Anole Lizard (*Anolis carolinensis*). *Ethology* **112**, 370–378.
- Brandt, Y. (2003). Lizard threat display handicaps endurance. *Proc. R. Soc. B Biol. Sci.* **270**, 1061–1068.
- Briffa, M. & Elwood, R.W. (2000). The power of shell rapping influences rates of eviction in hermit crabs. *Behav. Ecol.* **11**, 288 –293.
- Briffa, M. & Elwood, R.W. (2009). Difficulties remain in distinguishing between mutual and self-assessment in animal contests. *Anim. Behav.* **77**, 759–762.
- Briffa, M. & Elwood, R.W. (2010). Repeated measures analysis of contests and other dyadic interactions: problems of semantics, not statistical validity. *Anim. Behav.* **80**, 583–588.
- Briffa, M., Elwood, R.W. & Russ, J.M. (2003). Analysis of multiple aspects of a repeated signal: power and rate of rapping during shell fights in hermit crabs. *Behav. Ecol.* **14**, 60–65.

- Briffa, M. & Sneddon, L.U. (2010). Contest Behavior. In *Evolutionary Behavioral Ecology*: 246–265. Westneat, D.F. & Fox, C.W. (Eds). New York: Oxford University Press.
- Brown, S., Johnson, F. & Bottjer, S. (1993). Neurogenesis in adult canary telencephalon is independent of gonadal hormone levels. *J. Neurosci.* **13**, 2024–2032.
- Byers, J., Hebets, E. & Podos, J. (2010). Female mate choice based upon male motor performance. *Anim. Behav.* **79**, 771–778.
- Bywater, C.L. & Wilson, R.S. (2012). Is honesty the best policy? Testing signal reliability in fiddler crabs when receiver-dependent costs are high. *Funct. Ecol.*
- Canoine, V. & Gwinner, E. (2002). Seasonal differences in the hormonal control of territorial aggression in free-living European stonechats. *Horm. Behav.* **41**, 1–8.
- Clutton-Brock, T.H., Albon, S.D., Gibson, R.M. & Guinness, F.E. (1979). The logical stag: Adaptive aspects of fighting in red deer (*Cervus elaphus* L.). *Anim. Behav.* **27**, 211–225.
- Cox, R.M., Stenquist, D.S., Henningsen, J.P. & Calsbeek, R. (2009). Manipulating testosterone to assess links between behavior, morphology, and performance in the brown anole *Anolis sagrei*. *Physiol. Biochem. Zool.* **82**, 686–698.
- Crews, D. (1975). Effects of Different Components of Male Courtship Behavior on Environmentally Induced Ovarian Recrudescence and Mating Preferences in Lizard, *Anolis-Carolinensis*. *Anim. Behav.* **23**, 349–356.
- Dawkins, R. & Krebs, J.R. (1978). Animal Signals: Information or Manipulation. In *Behavioural Ecology*: 282–309. Krebs, J.R. & Davies, N.B. (Eds). Oxford: Blackwell.
- Decourcy, K.R. & Jenssen, T.A. (1994). Structure and use of male territorial headbob signals by the lizard *Anolis carolinensis*. *Anim. Behav.* **47**, 251–262.
- Dial, K.P., Greene, E. & Irschick, D.J. (2008). Allometry of behavior. *Trends Ecol. Evol.* **23**, 394–401.
- Edwards, J.R. & Lailvaux, S.P. (2012). Display Behavior and Habitat Use in Single and Mixed Populations of *Anolis carolinensis* and *Anolis sagrei* Lizards. *Ethology* **118**, 494–502.
- Endler, J.A. (1980). Natural Selection on Color Patterns in *Poecilia reticulata*. *Evolution* **34**, 76–91.
- Enquist, M. & Leimar, O. (1983). Evolution of fighting behaviour: Decision rules and assessment of relative strength. *J. Theor. Biol.* **102**, 387–410.

- Fitzstephens, D.M. & Getty, T. (2000). Colour, fat and social status in male damselflies, *Calopteryx maculata*. *Anim. Behav.* **60**, 851–855.
- Garland, T. & Else, P.L. (1987). Seasonal, sexual, and individual variation in endurance and activity metabolism in lizards. *Am. J. Physiol. - Regul. Integr. Comp. Physiol.* **252**, R439–R449.
- Garland, T., Hankins, E. & Huey, R.B. (1990). Locomotor Capacity and Social Dominance in Male Lizards. *Funct. Ecol.* **4**, 243–250.
- Getty, T. (1998). Handicap signalling: when fecundity and viability do not add up. *Anim. Behav.* **56**, 127–130.
- Goeritz, F., Quest, M., Wagener, A., Fassbender, M., Broich, A., Hildebrandt, T.B., Hofmann, R.R. & Blottner, S. (2003). Seasonal timing of sperm production in roe deer: interrelationship among changes in ejaculate parameters, morphology and function of testis and accessory glands. *Theriogenology* **59**, 1487–1502.
- Grafen, A. (1990). Sexual selection unhandicapped by the Fisher process. *J. Theor. Biol.* **144**, 473–516.
- Greenberg, B. & Noble, G.K. (1944). Social behavior of the American chameleon (*Anolis carolinensis* Voigt). *Physiol. Zool.* **17**, 392–439.
- Hack, M.A. (1997). Assessment strategies in the contests of male crickets, *Acheta domesticus*(L.). *Anim. Behav.* **53**, 733–747.
- Hau, M. (2007). Regulation of male traits by testosterone: implications for the evolution of vertebrate life histories. *BioEssays* **29**, 133–144.
- Henningsen, J.P. & Irschick, D.J. (2012). An experimental test of the effect of signal size and performance capacity on dominance in the green anole lizard. *Funct. Ecol.* **26**, 3–10.
- Herrel, A. & Gibb, A.C. (2006). Ontogeny of performance in vertebrates. *Physiol. Biochem. Zool. Pbz* **79**, 1–6.
- Herrel, A., De Gruaw, E. & Lemos-Espinal, J.A. (2001). Head Shape and Bite Force Performance in Xenosaurid Lizards. *J. Exp. Zool.* **29**, 101–107.
- Herrel, A., Spithoven, L., Van Damme, R. & De Vree, F. (1999). Sexual dimorphism of head size in *Gallotia galloti*: testing the niche divergence hypothesis by functional analyses. *Funct. Ecol.* **13**, 289–297.
- Hof, D. & Hazlett, N. (2010). Low-amplitude song predicts attack in a North American wood warbler. *Anim. Behav.* **80**, 821–828.

- Holmes, M., Bartrem, C. & Wade, J. (2007). Androgen dependent seasonal changes in muscle fiber type in the dewlap neuromuscular system of green anoles. *Physiol. Behav.* **91**, 601–608.
- Holmes, M. & Wade, J. (2005). Testosterone regulates androgen receptor immunoreactivity in the copulatory, but not courtship, neuromuscular system in adult male green anoles. *J. Neuroendocrinol.* **17**, 560–569.
- Hughes, M. (1996). Size assessment via a visual signal in snapping shrimp. *Behav. Ecol. Sociobiol.* **38**, 51–57.
- Hunt, J., Brooks, R., Jennions, M.D., Smith, M.J., Bentsen, C.L. & Bussiere, L.F. (2004). High-quality male field crickets invest heavily in sexual display but die young. *Nature* **432**, 1024–1027.
- Husak, J.F., Fox, S.F., Lovern, M.B. & Van Den Bussche, R.A. (2006). Faster lizards sire more offspring: Sexual selection on whole-animal performance. *Evolution* **60**, 2122–2130.
- Husak, J.F., Henningsen, J.P., VanHooydonck, B. & Irschick, D.J. (In Press). A Functional Approach to Studying Costs of Animal Signals. In *Animal Signaling: Functional and Evolutionary Perspectives*. Irschick, D.J., Briffa, M. & Podos, J. (Eds). Hoboken, NJ: John Wiley and Sons.
- Husak, J.F. & Irschick, D.J. (2009). Steroid use and human performance: Lessons for integrative biologists. *Integr. Comp. Biol.* **49**, 354–364.
- Husak, J.F., Irschick, D.J., Meyers, J.J., Lailvaux, S.P. & Moore, I. (2007). Hormones, sexual signals, and performance of green anole lizards (*Anolis carolinensis*). *Horm. Behav.* **52**, 360–367.
- Husak, J.F., Lappin, A.K. & Van Den Bussche, R. (2009). The fitness advantage of a high-performance weapon. *Biol. J. Linn. Soc.* **96**, 840–845.
- Husak, J.F., Ribak, G., Wilkinson, G.S. & Swallow, J.G. (2011). Compensation for exaggerated eye stalks in stalk-eyed flies (Diopsidae). *Funct. Ecol.* **25**, 608–616.
- Huyghe, K., Husak, J.F., Moore, I.T., Vanhooydonck, B., Damme, R.V., Molina-Borja, M. & Herrel, A. (2010). Effects of testosterone on morphology, performance and muscle mass in a lizard. *J. Exp. Zool. Part Ecol. Genet. Physiol.* **313A**, 9–16.
- Huyghe, K., Vanhooydonck, B., Scheers, H., Molina-Borja, M. & Van Damme, R. (2005). Morphology, performance and fighting capacity in male lizards, *Gallotia galloti*. *Funct. Ecol.* **19**, 800–807.
- Irschick, D.J., Herrel, A., Vanhooydonck, B. & Van Damme, R. (2007). A functional approach to sexual selection. *Funct. Ecol.* **21**, 621–626.

- Irschick, D.J., Meyers, J., Husak, J.F. & Le Galliard, J.F. (2008). How does selection operate on whole-organism functional performance capacities? A review and synthesis. *Evol. Ecol. Res.* **10**, 177–196.
- Irschick, D.J., Ramos, M., Buckley, C., Elstrott, J., Carlisle, E., Lailvaux, S.P., Bloch, N., Herrel, A. & Vanhooydonck, B. (2006). Are morphology-performance relationships invariant across different seasons? A test with the green anole lizard (*Anolis carolinensis*). *Oikos* **114**, 49–59.
- Jakob, E., Marshall, S.D. & Uetz, G.W. (1996). Estimating fitness components: a comparison of body condition indices. *Oikos* **77**, 61–67.
- Jenssen, T.A., Decourcy, K.R. & Congdon, J.D. (2005). Assessment in contests of male lizards (*Anolis carolinensis*): how should smaller males respond when size matters? *Anim. Behav.* **69**, 1325–1336.
- Jenssen, T.A., Greenberg, N. & Novde, K.A. (1995). Behavioral profile of free-ranging male lizards, *Anolis carolinensis*, across breeding and post-breeding seasons. *Herpetol. Monogr.* **9**, 41–62.
- Jenssen, T.A., Lovern, M.B. & Congdon, J.D. (2001). Field-testing the protandry-based mating system for the lizard, *Anolis carolinensis*: does the model organism have the right model? *Behav. Ecol. Sociobiol.* **50**, 162–172.
- Jenssen, T.A., Orrell, K.S. & Lovern, M.B. (2000). Sexual dimorphisms in aggressive signal structure and use by a polygynous lizard, *Anolis carolinensis*. *Copeia* **2000**, 140–149.
- John-Alder, H.B. (1984). Seasonal variations in activity, aerobic energetic capacities, and plasma thyroid hormones (T3 and T4) in an iguanid lizard. *J. Comp. Physiol. B* **154**, 409–419.
- Johnstone, R.A. (1997). The evolution of animal signals. In *Behavioural Ecology: an evolutionary approach*: 155–178. Krebs, J.R. & Davies, N.B. (Eds). Oxford: Blackwell.
- Jonart, L.M., Hill, G.E. & Badyaev, A.V. (2007). Fighting ability and motivation: determinants of dominance and contest strategies in females of a passerine bird. *Anim. Behav.* **74**, 1675–1681.
- Karsten, K.B., Andriamandimbarisoa, L.N., Fox, S.F. & Raxworthy, C.J. (2009). Sexual selection on body size and secondary sexual characters in 2 closely related, sympatric chameleons in Madagascar. *Behav. Ecol.* **20**, 1079–1088.
- Ketterson, E.D., Nolan, V., Castro, J., Buerkle, C.A., Clotfelter, E., Grindstaff, J.L. & Jones, K.J. (2001). Testosterone, phenotype and fitness: a research program in evolutionary behavioral endocrinology. In *Avian Endocrinology*: 19–40. New Delhi: Narosa.

- Korzan, W.J., Summers, T.R., Ronan, P.J. & Summers, C.H. (2000). Visible Sympathetic Activity as a Social Signal in *Anolis carolinensis*: Changes in Aggression and Plasma Catecholamines. *Horm. Behav.* **38**, 193–199.
- Kotiaho, J.S. (2000). Testing the assumptions of conditional handicap theory: costs and condition dependence of a sexually selected trait. *Behav. Ecol. Sociobiol.* **48**, 188–194.
- Kotiaho, J.S. (2001). Costs of sexual traits: a mismatch between theoretical considerations and empirical evidence. *Biol. Rev.* **76**, 365–376.
- Kotiaho, J.S., Alatalo, R.V., Mappes, J., Nielsen, M.G., Parri, S. & Rivero, A. (1998). Energetic costs of size and sexual signalling in a wolf spider. *Proc. R. Soc. Lond. Ser. B-Biol. Sci.* **265**, 2203–2209.
- Laidre, M.E. (2007). Vulnerability and reliable signaling in conflicts between hermit crabs. *Behav. Ecol.* **18**, 736–741.
- Lailvaux, S.P., Gilbert, R.L. & Edwards, J.R. (2012). A Performance-Based Cost to Honest Signalling in Male Green Anole Lizards (*Anolis Carolinensis*). *Proc. R. Soc. B Biol. Sci.*
- Lailvaux, S.P., Herrel, A., VanHooydonck, B., Meyers, J.J. & Irschick, D.J. (2004). Performance capacity, fighting tactics and the evolution of life-stage male morphs in the green anole lizard (*Anolis carolinensis*). *Proc. R. Soc. Lond. Ser. B-Biol. Sci.* **271**, 2501–2508.
- Lailvaux, S.P. & Irschick, D.J. (2006a). A functional perspective on sexual selection: insights and future prospects. *Anim. Behav.* **72**, 263–273.
- Lailvaux, S.P. & Irschick, D.J. (2006b). No evidence for female association with high-performance males in the green anole lizard, *Anolis carolinensis*. *Ethology* **112**, 707–715.
- Lailvaux, S.P. & Irschick, D.J. (2007). The evolution of performance-based male fighting ability in Caribbean *Anolis* lizards. *Am. Nat.* **170**, 573–586.
- Lappin, A.K. & Husak, J.F. (2005). Weapon performance, not size, determines mating success and potential reproductive output in the collared lizard (*Crotaphytus collaris*). *Am. Nat.* **166**, 426–436.
- Lindström, L., Ahtiainen, J.J., Mappes, J., Kotiaho, J.S., Lyytinen, A. & Alatalo, R.V. (2006). Negatively condition dependent predation cost of a positively condition dependent sexual signalling. *J. Evol. Biol.* **19**, 649–656.
- Losos, J.B. (2009). *Lizards in an Evolutionary Tree: Ecology and Adaptive Radiation of Anoles*. Berkeley: University of California Press.

- Losos, J.B. & Chu, L.R. (1998). Examination of factors potentially affecting dewlap size in Caribbean anoles. *Copeia* **2**, 430–438.
- Losos, J.B., Creer, D.A. & Schulte II, J.A. (2002). Cautionary comments on the measurement of maximum locomotor capabilities. *J. Zool.* **258**, 57–61.
- Lovern, M.B., Holmes, M.M., Fuller, C.O. & Wade, J. (2004). Effects of testosterone on the development of neuromuscular systems and their target tissues involved in courtship and copulation in green anoles (*Anolis carolinensis*). *Horm. Behav.* **45**, 295–305.
- Lovern, M.B., Jenssen, T.A., Orrell, K.S. & Tuchak, T. (1999). Comparisons of temporal display structure across contexts and populations in male *Anolis carolinensis*: Signal stability or lability? *Herpetologica* **55**, 222–234.
- Lovern, M.B., McNabb, F.M.A. & Jenssen, T.A. (2001). Developmental effects of testosterone on behavior in male and female green anoles (*Anolis carolinensis*). *Horm. Behav.* **39**, 131–143.
- Marler, C.A. & Moore, M.C. (1988). Evolutionary costs of aggression revealed by testosterone manipulations in free-living male lizards. *Behav. Ecol. Sociobiol.* **23**, 21–26.
- Maynard-Smith, J. & Harper, D. (2003). *Animal Signals*. Oxford: Oxford University Press.
- McCann, T.S. (1981). Aggression and sexual activity of male Southern elephant seals, *Mirounga leonina*. *J. Zool.* **195**, 295–310.
- McCullough, E.L. & Tobalske, B.W. (2013). Elaborate horns in a giant rhinoceros beetle incur negligible aerodynamic costs. *Proc. R. Soc. B Biol. Sci.* **280**.
- McMann, S. (1993). Contextual signalling and the structure of dyadic encounters in *Anolis carolinensis*. *Anim. Behav.* **46**, 657–668.
- McMillan, D.M. & Irschick, D.J. (2010). An experimental test of predation and competition pressures on the green anole lizard (*Anolis carolinensis*) in varying structural habitats. *J. Herpetol.* **44**, 272–278.
- Meyers, J.J., Herrel, A. & Birch, J. (2002). Scaling of morphology, bite force, and feeding kinematics in an iguanian and a scleroglossan lizard. In *Topics in Functional and Ecological Vertebrate Morphology*: 47–62. Aerts, P., D'aout, K., Herrel, A. & Van Damme, R. (Eds). Maastricht, Netherlands: Shaker Publishing B.V.
- Møller, A.P. & Nielsen, J.T. (1997). Differential predation cost of a secondary sexual character: sparrowhawk predation on barn swallows. *Anim. Behav.* **54**, 1545–1551.

- Moodie, G.E.E. (1972). Heredity - Abstract of article: Predation, natural selection and adaptation in an unusual threespine stickleback. *Heredity* **28**, 155–167.
- Nakagawa, S. (2004). A farewell to Bonferroni: the problems of low statistical power and publication bias. *Behav Ecol* **15**, 1044–1045.
- Nicholson, K.E., Harmon, L.J. & Losos, J.B. (2007). Evolution of Anolis Lizard Dewlap Diversity. *Plos One* **2**, e274.
- O'Connor, J.L., McBrayer, L.D., Higham, T.E., Husak, J.F., Moore, I.T. & Rostal, D.C. (2011). Effects of Training and Testosterone on Muscle Fiber Types and Locomotor Performance in Male Six-Lined Racerunners (*Aspidoscelis sexlineata*). *Physiol. Biochem. Zool.* **84**, 394–405.
- Oliveira, R.F. (2004). Social modulation of androgens in vertebrates: mechanisms and function. *Adv. Study Behav.* **34**, 165–239.
- Ord, T.J. & Martins, E.P. (2006). Tracing the origins of signal diversity in anole lizards: phylogenetic approaches to inferring the evolution of complex behaviour. *Anim. Behav.* **71**, 1411–1429.
- Orrell, K.S., Congdon, J.D., Jenssen, T.A., Michener, R.H. & Kunz, T.H. (2004). Intersexual differences in energy expenditure of *Anolis carolinensis* lizards during breeding and postbreeding seasons. *Physiol. Biochem. Zool. Pbz* **77**, 50–64.
- Oufiero, C.E. & Garland, T.J. (2007). Evaluating performance costs of sexually selected traits. *Funct. Ecol.* **21**, 676–689.
- Panhuis, T.M. & Wilkinson, G.S. (1999). Exaggerated male eye span influences contest outcome in stalk-eyed flies (Diopsidae). *Behav. Ecol. Sociobiol.* **46**, 221–227.
- Parker, G.A. & Stuart, R.A. (1976). Animal Behavior as a Strategy Optimizer: Evolution of Resource Assessment Strategies and Optimal Emigration Thresholds. *Am. Nat.* **110**, 1055–1076.
- Payne, R.J.H. (1998). Gradually escalating fights and displays: the cumulative assessment model. *Anim. Behav.* **56**, 651–662.
- Peig, J. & Green, A.J. (2010). The paradigm of body condition: a critical reappraisal of current methods based on mass and length. *Funct. Ecol.* **24**, 1323–1332.
- Perry, G., Levering, K., Girard, I. & Garland, T. (2004). Locomotor performance and dominance in male *Anolis cristatellus*. *Anim. Behav.* **67**, 37–47.
- R Development Core Team. (2008). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

- Rimmer, D.M., Saunders, R.L. & Paim, U. (1985). Effects of temperature and season on the position holding performance of juvenile Atlantic salmon (*Salmo salar*). *Can. J. Zool.* **63**, 92–96.
- Robson, M.A. & Miles, D.B. (2000). Locomotor performance and dominance in male tree lizards, *Urosaurus ornatus*. *Funct. Ecol.* **14**, 338–344.
- Rohwer, S. (1977). Status signalling in Harris' sparrows: some experiments in deception. *Behaviour* **61**, 107–129.
- Ryan, M.J. (1980). Female mate choice in a neotropical frog. *Science* **209**, 523–525.
- Ryan, M.J., Tuttle, M.D. & Rand, A.S. (1982). Bat predation and sexual advertisement in a neotropical anuran. *Am. Nat.* **119**, 136–139.
- Searcy, W.A. & Nowicki, S. (2005). *The Evolution of Animal Communication: Reliability and Deception in Signaling Systems.*, Monographs in Behavior and Ecology. Princeton: Princeton University Press.
- Sidor, C.A. & Blackburn, D.G. (1998). Effects of testosterone administration and castration on the forelimb musculature of male leopard frogs, *Rana pipiens*. *J. Exp. Zool.* **280**, 28–37.
- Smith, G.T., Brenowitz, E.A., Beecher, M.D. & Wingfield, J.C. (1997). Seasonal changes in testosterone, neural attributes of song control nuclei, and song structure in wild songbirds. *J. Neurosci.* **17**, 6001–6010.
- Sneddon, L.U., Huntingford, F.A., Taylor, A.C. & Orr, J.F. (2000). Weapon Strength and Competitive Success in the Fights of Shore Crabs (*Carcinus maenas*). *J. Zool.* **250**, 397–403.
- Sokal, R.R. & Rohlf, F.J. (1995). *Biometry the principles and practice of statistics in biological research*. Third. New York: W.J. Freeman and Company.
- Soma, K.K. & Wingfield, J.C. (2001). Dehydroepiandrosterone in Songbird Plasma: Seasonal Regulation and Relationship to Territorial Aggression. *Gen. Comp. Endocrinol.* **123**, 144–155.
- Summers, C.H. & Greenberg, N. (1994). Somatic Correlates of Adrenergic Activity during Aggression in the Lizard, *Anolis carolinensis*. *Horm. Behav.* **28**, 29–40.
- Taigen, T.L. & Wells, K.D. (1985). Energetics of vocalization by an anuran amphibian (*Hyla versicolor*). *J. Comp. Physiol. B* **155**, 163–170.
- Thornhill, R. (1981). *Panorpa* (Mecoptera: Panorpidae) Scorpionflies: Systems for Understanding Resource-Defense Polygyny and Alternative Male Reproductive Efforts. *Annu. Rev. Ecol. Syst.* **12**, 355–386.

- Tibbetts, E.A. & Lindsay, R. (2008). Visual signals of status and rival assessment in *Polistes dominulus* paper wasps. *Biol. Lett.* **4**, 237–239.
- Tokarz, R.R. (2002). An Experimental Test of the Importance of the Dewlap in Male Mating Success in the Lizard *Anolis sagrei*. *Herpetologica* **58**, 87–94.
- Tokarz, R.R., Paterson, A.V. & McMann, S. (2003). Laboratory and field test of the functional significance of the male's dewlap in the lizard *Anolis sagrei*. *Copeia* **3**, 502–511.
- Tokarz, R.R., Paterson, A.V. & McMann, S. (2005). Importance of dewlap display in male mating success in free-ranging brown anoles (*Anolis sagrei*). *J. Herpetol.* **39**, 174–177.
- Trivers, R.L. (1976). Sexual Selection and Resource-Accruing Abilities in *Anolis garmani*. *Evolution* **30**, 253–269.
- Vanhooydonck, B., Herrel, A., Van Damme, R. & Irschick, D.J. (2005a). Does dewlap size predict male bite performance in Jamaican *Anolis* lizards? *Funct. Ecol.* **19**, 38–42.
- Vanhooydonck, B., Herrel, A., Van Damme, R., Meyers, J. & Irschick, D.J. (2005b). The relationship between dewlap size and performance changes with age and sex in a Green Anole (*Anolis carolinensis*) lizard population. *Behav. Ecol. Sociobiol.* **59**, 157–165.
- Warner, R.R., Robertson, D.R. & Leigh, E.G. (1975). Sex Change and Sexual Selection. *Science* **190**, 633–638.
- Whitham, T.G. (1986). Cost of Benefits of Territoriality: Behavioral and Reproductive Release by Competing Aphids. *Ecology* **67**, 139.
- Wikelski, M. & Trillmich, F. (1997). Body Size and Sexual Size Dimorphism in Marine Iguanas Fluctuate as a Result of Opposing Natural and Sexual Selection: An Island Comparison. *Evolution* **51**, 922–936.
- Wilkinson, G.S., Kahler, H. & Baker, R.H. (1998). Evolution of female mating preferences in stalk-eyed flies. *Behav. Ecol.* **9**, 525–533.
- Wingfield, J.C. & Farner, D.S. (1975). Determination of 5 steroids in avian plasma by radioimmunoassay and competitive-protein-binding. *Steroids* **26**, 311–327.
- Wingfield, J.C., Hegner, R.E., Dufty, A.M. & Ball, G.F. (1990). The “challenge hypothesis”: theoretical implications for patterns of testosterone secretion, mating systems and breeding strategies. *Am. Nat.* **136**, 829–846.
- Zahavi, A. (1975). Mate Selection - Selection for a Handicap. *J. Theor. Biol.* **53**, 205–214.